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In silico analysis of cubebinol for evaluating its efficiency against menacing respiratory ailments

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Over the recent decade a survey states that the advent of respiratory diseases had took a rapid transmittance and transformation rate. The mortality and the morbidity rates were also exorbitant. *Piper cubeba* is one of the traditional plant species belongs to Piperacea family, which possess good antibacterial activity. The plant comprise of several phytocomponent one among which is cubebinol, whose specific activities have not been much explored. Hence it is subjected in this research and its antibacterial efficiency is investigated through virtual screening technique. Techniques like Auto dock, Discovery studio, Pymol are evolved in the investigation to know the unknown nature of the phytocomponent by analyzing its binding affinity along with the major respiratory disease causing organism's macromolecules. Thus it manifests the efficiency and the potency of the plant phytocomponent, which is found to be better than that of the readily available and commercially consumed drug molecules. By both the pharmacokinetic test as well as the docking validation we found that the docked ligand compound cubebinol is a potent drug against several fatal bacterial respiratory diseases.

Keywords: Cubebinol, Molecular docking, Phytochemical, *Piper cubeba*, Respiratory diseases

A report states that nearly 100 million people suffer from respiratory track diseases and nearly 4 million people die early due to these respiratory ailments. The major dreadful respiratory ailments reported to be Asthma, Chronic obstructive pulmonary disease, acute respiratory infection, cystic fibrosis, tuberculosis, and lung cancer [1]. Asthma is considered to be as one of the life threatening disease which has several diagnostic problems and also its unveils certain age specific characteristics .In mild asthma there is no detectable changes and no obvious clinical changes in airflow obstruction whereas in moderate and severe asthma will have a clear evidence of airway obstruction[2]. *Chlamydia pneumonia*, *Mycoplasma pneumonia* and *Staphylococcus pneumonia* are the major organisms responsible for transmission of asthma. The next stated respiratory disease COPD is one of the dreadful disease where chronic airway obstruction occurs and there is no way of reversing back to its normal form that is still now there is no proper curative is found for COPD[3]. Acute respiratory infections hold a position in causing mortality and morbidity in several countries, common infections of respiratory track are bronchitis, laryngitis, cold and cough, diphtheria, pertussis or whooping cough. Major causative agents are *Streptococcus pneumonia* and *Haemophillus influenza*. Cystic fibrosis is considered to be as one of the dreadful diseases which cause mucous plugging due to autosomal recessiveness. Early mild symptoms of cystic fibrosis are not well expressed thus they couldn't be diagnosed in early stages[4]. *Pseudomonas aeruginosa* is the major causative organism of cystic fibrosis. Tuberculosis prevailed its origin in 1700 and early 1800 it started its spread in several countries and been a major cause for morbidity mycoplasma tuberculosis is the major causative agent of this malignant disease[5,6]. The leading cancer deaths is due to the lung cancer worldwide, it processes high morbidity rate due to the inefficiency to detect them in the early stages they could not visibly show their effect in initial stages[7]. Plant phytocomponents are said to possess good antibacterial property. For example, Vetrivel et al. stated that plant phytocomponents like *Tellimagrandin-II* from *S.aromaticum* and *O-Demethyl-demethoxycurcumin* from *Curcuma longa* possessed a good efficiency against the respiratory disease. This was confirmed with the docking results provided by macromolecule and the plant phytocomponents[8]. Also Baptista et al. reports that α-cubebin, curcumin, hydroquinone etc. were chosen as the natural phytocomponents and they were allowed to dock against the bacterial respiratory viral protein which is

responsible to cause tuberculosis hence its antimicrobial activity was investigated. The natural phytocomponents produces good binding score thus its efficiency was proven through molecular docking studies[9]. As cubebin and cubebinol possess similar physiochemical characteristics the later was subjected in this study to understand its efficiency against menacing respiratory diseases.

Piper cubeba belongs to the piperacea family, more than 700 piper species evolve around the world over which *Piper cubeba*'s several biological activities have not been explored much, it is mostly harvested from the region Java and Borneo hence it is termed as Java pepper. It is commonly used for conventional medicinal treatments of respiratory diseases like common cold, asthma then several sexually transmitted bacterial diseases like gonorrhea, syphilis, also several abdominal disorders like abdominal pain, diarrhea, enteritis and dysentery. Hence it shows that the plant component possesses a good antibacterial activity. *Piper cubeba* is comprised of several flavonoids, alkaloids and phytocomponent. Cubebinol a phytocomponent present in *Piper cubeba* which has a melting point 92°C. Cubebinol is formed after the formation of dihydrocubebinic ether. Dihydrocubebinic ether is reduced to a monohydroxy compound (cubebinol) where a ring opens by adding sodium along with ethanol[10].

Virtual screening is one of the best, efficient time and cost saving process by which we can easily analyze the nature of the protein and the ligand or the drug like compound. The virtual screening helps the researchers to promote a different pathway for discovering new drug compound. Molecular docking plays a vital role in computer aided drug designing and delivery and also in structural molecular biology. By performing this study we could easily determine the predominant binding modes of the protein ligand complex by analyzing its binding score[11]. It is considered to be as one of the efficient ways for the researchers in order to study the preprocessing nature of the drug molecule by analyzing its interactive nature. The protein structures could be easily predicted using the instrumentation techniques like NMR and X-ray crystallography yet molecular docking is most widely used as a lead optimization tool to figure out the drug ability of a compound and its specificity towards the receptors binding site. Further simulation studies could be performed to interrogate the docked complex nature inside the human[12]. By using Auto dock tool we could easily analyze the close proximity of the protein and ligand molecule after the pre study of the compounds we could understand the binding affinity of the molecules then after analyzing it we could perform the *in vivo* or *in vitro* studies easily [13-15]. As proposed in literatures the antibacterial efficiency of a phytocomponent from *Piper cubeba* will be estimated against several respiratory diseases by comparing it with the already commercially available drugs provided for those diseases through virtual screening process.

Experimental Section

System requirement

As stated in the auto dock manual the preliminary requirement were followed by using the system with properties: systems inbuilt memory is 8.00 GB RAM , 11th Gen Intel(R) Core(TM) i3-1115G4 @ 3.00GHz 3.00 GHz, and the type stated to be 64-bit operating system, x64-based processor.

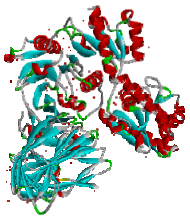
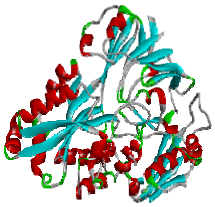
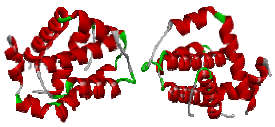
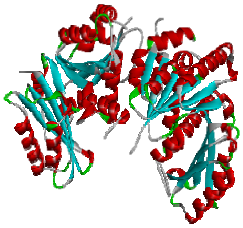
Protein preference

Macromolecule preference is one of the most vital part in the molecular dynamic study. Usually the detailed molecular structure of the protein complex is chosen from Protein Data Bank database it is one of the huge and best source of macromolecule database[16]. Based upon the examination underwent in the literatures five different macromolecules were chosen. With this link (PDB; http://www.rcsb.org/pdb/) the macromolecule were able to be downloaded. It provides the clear cut data regarding the protein molecule like its origin of organism its method macromolecule nature etc. Also the complete details of macromolecule will be provided by PDB[17]. The following five protein molecules of mortal respiratory diseased protein have been chosen for this experimental analysis. From Table 1 we can get the complete details of the protein molecules of the 5 respective respiratory diseases their pdb id along with their name is provided and their 3D structure is also provided.

Ligand preference

The ligand compound chosen was the phytocomponent from *Piper cubeba* plant. Cubebinol ligand was chosen because of its efficiency still remains unexplored [18]. As it remains unexplored in order to have a proof for performing this study a similar compound cubebin's physicochemical property was explored with the help of software. The

Table 1 — List of macromolecules used in the experimental analysis

Disease	PDB ID	Name of protein	Image
Asthma	6LNW	Crystal structure of accessory secretory protein 1,2 and 3 in Streptococcus pneumoniae	
Chronic obstructive pulmonary disease	7OFW	Nontypeable Haemophillus influenzae SapA in complex with heme	
Cystic Fibrosis	1CKW	*Cystic fibrosis transmembrane conductance regulator: solution structures of peptides based on the phe508 region, the most common site of disease-causing delta-f508 mutation*	
Lung Cancer	4LVT	*Bcl_2-Navitoclax (ABT-263) Complex*	
Tuberculosis	6YM1	Mycobacterium tuberculosis FtsZ in complex with GDP	

molecular weight of cubebin is determined to be 356.37 with the Log P value of 2.51. Cubebin structure is composed of 20 carbon atoms in which it has 3 chiral carbon atoms. It is determined that there is no sp hybridized carbons atoms in cubebin, the number of sp^2 hybridized carbon atoms present in cubebin were found to be 12 and the number of sp^3 hybridized carbon is found to be 8. The number of rotatable bonds present in cubebin was determined to be 4. Similarly the compound cubebinol's physicochemical property was evaluated in the software and which is given in Table 2, it showed similar property nature as cubebinol's molecular weight was found to be 338.36 with Log P value of 2.93. The structure of cubebinol is composed of 20 carbon atoms in which it has 1 chiral carbon atom. It is determined that there is no sp hybridized carbons atoms in cubebinol, the number of sp^2

Table 2 — Cubebinol's physiochemical property obtained from RDKit: Open-Source Cheminformatics software compared with cubebin

Property name	Property value	
	Cubebinol	Cubebin
Molecular weight (g/mol)	338.36	356.37
Log P	2.93	2.15
Topological polar surface area (Å^2)	57.15	66.38
Number of hydrogen bond acceptors	5	6
Number of hydrogen bond donors	1	1
Number of carbon atoms	20	20
Number of heavy atoms	25	26
Number of heteroatoms	5	6
Number of nitrogen atoms	0	0
Number of sulfur atoms	0	0
Number of chiral carbon atoms	1	3

hybridized carbon atoms present in cubebin was found to be 14 and the number of sp^3 hybridized carbon is found to be 6. The number of rotatable bonds present in cubebin was determined to be 4. On comparison, it shows only minor difference in both cubebin and cubebinol's physicochemical property. Hence the compound cubebinol was chosen in this study. The SEM image of cubebinol (green synthesised from piper cubeba and nanotised) is shown clearly in Fig. 1. The SEM micrograph shows spherical morphology of cubebinol particles with the size range of 74.6 nm, as the overall average size range of the particle. The ligand molecules chemical structure was downloaded from a plant database termed IMPAAT (https://cb.imsc.res.in/imppat/phytochemical-detailed-page/IMPHY000529) which consist of the structures and complete details of plant phytocomponents[19]. The phytocomponent cubebinol's chemical structure, physicochemical and drug likelinessproperties were explored in databases RDKit: Open-Source Cheminformatics Software (http://www.rdkit.org/), the details obtained for cubebinol were listed in the given below Table 2. The commercially consumable drugs for the following respiratory illness were referred in the literatures and TTD Therapeutic Target Database https://db.idrblab.net/ttd/, then they were downloaded from PubChemhttps://pubchem.ncbi.nlm.nih.gov/ they were downloaded in sdf format and they were converted into pdbqt format which is the preferred format for docking using open bable software[20].

ADMET properties and drug-likeliness property

The main quality of a drug is determined only by its adsorption, distribution, metabolism, excretion and toxicity properties hence the properties are evaluated for a drug molecule before converting into a drug molecule for clinical evaluation. Tools like AdmetSAR (http://www.admetexp.org) and Swiss ADME (http://www.swissadme.ch/) are employed in this evaluation process to bring out the ADMET properties of the ligand molecule[21]. Drug likeliness property of a novel developed drug molecule should follow the following 5 rule

- Lipinski's rule of 5
- Ghose rule
- Veber rule
- Egan rule
- GSK 4/400 rule

Thus these five rules are mainly used to determine the drug likeliness property of a newly developed

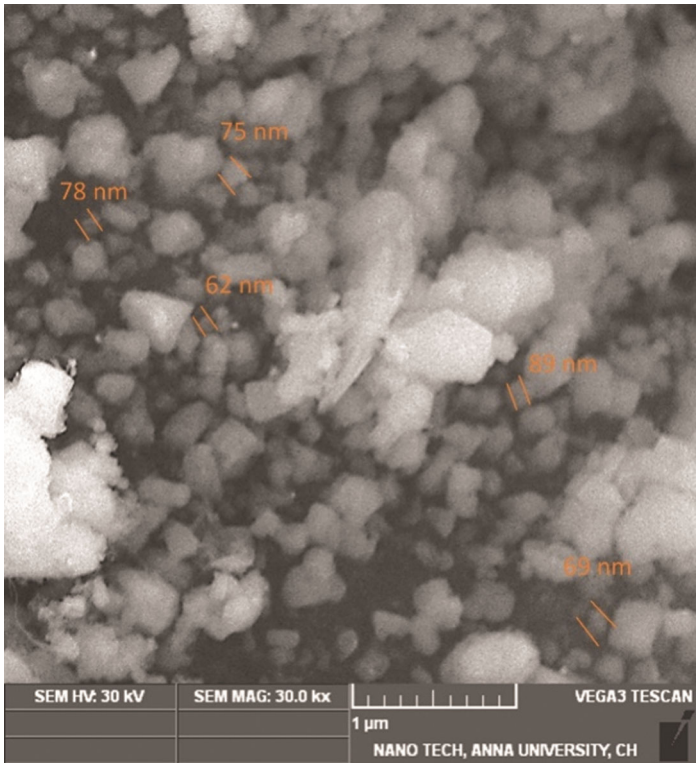


Fig. 1 — SEM micrograph of cubebinol

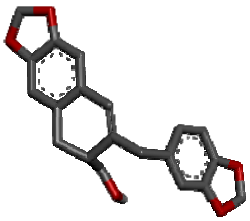
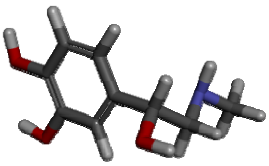
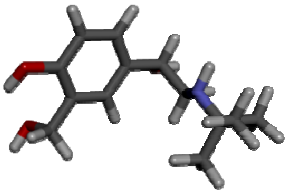
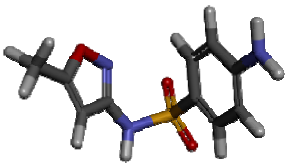
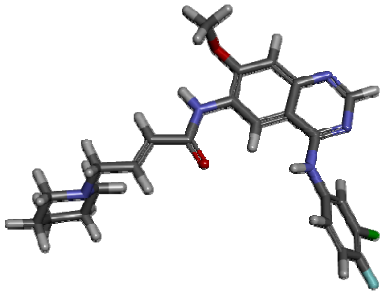
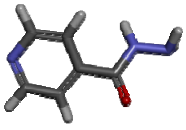
drug molecule. These properties were evaluated using the database RDKit: Open-Source Cheminformatics Software (http://www.rdkit.org/) for cubebinol and commercially used drugs.

Molecular docking

Protein and ligand preparation

After choosing the protein molecule their active sites were predicted through PAR-3D (http://sunserver.cdfd.org.in:8080/protease/PAR_3D/index.html). The excess compounds present in the protein complex is removed along with the water molecule, because the water molecule affects the thermodynamics and kinetics of the protein ligand complex thus they are eliminated prior to the docking studies. The downloaded cubebinol in SDF format is converted into PDBQT format using open bable software. And the polar hydrogens were added to the macromolecule. Polar hydrogens are hydrogens that are bonded to electronegative atoms like oxygen and nitrogen. From Table 3, we could get the clear three dimensional image of the ligands chosen along with its ID and number of rotatable bonds present in the ligand.

Table 3 — Details regarding the ligand molecule

Ligand name	Chemspider ID	Image	No. of rotatble bonds
Cubebinol	CASID_22296-77-1		4
Epinephrine	CID:5816		3
Amoxicillin	CID:33613		4
Sulfamethoxazole	CID:5329		3
Etoposide	CID:36462		5
Isoniazid	CID:3767		1

Initialization of molecules

Each of the five protein molecules were made into separate folders and opened in the workspace separately along with the ligand molecule in AUTODOCK 4.2 software to analyze its binding nature. After addition of the protein molecule the Kollman charges and the gasterstrier charges were added to the each protein molecules before performing docking, where the Kollman charges determines the electrostatic potential of each amino acid. The gasterstrier charges were added to the macromolecule. The torsion tree of the ligand molecules were detected along with the roots.

Auto grid

After initialization of the molecules the grid parameters of the molecules are to be set. Precomputation of potential grid maps is provided by the Auto grid. The grid is set as per the complete accommodation of the active site along with the ligand molecule is ensured after setting the Auto grid parameters the output file is saved as GPF format , this file is used as the input and the Auto grid program is executed thus we get the resultant file to be in GLG file format[22].

Autodock

After performance of Auto grid, Auto dock is to be performed in which the macromolecule is to be selected in which the docking parameters must be set in the genetic algorithm. With the minimum run number of 50 Lamarckian algorithm was set along with 300 as their population and 2.5 million to be as their energy evaluation and 27000 as their number of generation with their cluster tolerance to be as 2.0 Å. Then the output file was saved to be in DPF format and then after the Auto dock run we would get the DLG file were we could determine the RMSD value, inhibitory constant binding affinity of the receptor and ligand molecule [23].

Visualization

Visualization is the process in which we could screen the image of the protein, ligand and receptor-ligand complex. It could be performed in the discovery studio tool where we could extract the 3 dimensional and 2 dimensional images of the receptor and ligand complex[24].

Results and Discussion

The ligand molecules were docked with five different macromolecules responsible for causing the dangerous respiratory ailments. The macromolecules are crystal structure of accessory secretory protein 1,2 and 3 in Streptococcus pneumoniae responsible for causing asthma, Nontypeable Haemophillus influenzae SapA in complex with heme- responsible for causing COPD, Cystic fibrosis transmembrane conductance regulator: solution structures of peptides based on the phe508 region, the most common site of disease-causing delta-f508 mutation-responsible for causing Cystic fibrosis, Bcl_2-Navitoclax (ABT-263) Complex-responsible for causing lung cancer and Mycobacterium tuberculosis and FtsZ in complex with GDP-responsible for causing tuberculosis among these mortal disease no disease has a permanent cure. As cubebinol, a phytocomponent from *Piper cubeba* states to has a great antibacterial property and it processes potency against several respiratory diseases. Hence its efficiency was tested against the life threatening diseases.

Docking validation

The macromolecules were prepared and docked against the ligand compound and in which their binding affinity, RMSD value, number of hydrogen bonds and the inhibitory constant of the docked complexes were estimated so that the efficiency of the ligand complex could be estimated. The strength of interaction between two molecules that is the macro molecule and the ligand is known to be as the binding affinity. The protein binding affinity acts as the key factor for enabling the protein interaction which would define the structure and function relationship between the ligand and macromolecule[25,26]. The strength of the drug binding with the receptor is described with the affinity. The ranking of the molecule is based on the scoring function and the scores given by the poses generated[27].

The ligand compounds were docked with the protein which is responsible for causing asthma and from Table 4, we could see that the macromolecule and the ligand compound cubebinol docked complex shows the good range of binding affinity[28]. The binding affinity obtained for the cubebinol and the asthma protein was found to be -8.56 kcal/mol which is a fine value than the binding affinity value of the standard drug and the mamcromolecule complex (-7.02 kcal/mol)[29]. The average deviation between the macromolecule and the ligand is measured using the RMSD value where the lower value determines close affinity. Here the macromolecule docked with cubebinol showed low RMSD value of 31.064 Å[30].

Table 4 — Docking validation of ligands docked with crystal structure of accessory secretory protein 1, 2 and 3 in *Streptococcus pneumoniae*

Protein name and pdb id & ligand	Amino acid involved in interaction	RMSD value (Å)	Binding energy (kcal/mol)	Inhibition onstant	No. of hydrogen bonds
Crystal structure of accessory secretory protein 1,2 and 3 in Streptococcus pneumoniae & 6LNW with Cubebinol	VAL127, ALA17, ARG28, PHE62, HIS58, GLN 56 and THR 20	31.064	-8.56	534.89 nM	5
Crystal structure of accessory secretory protein 1,2 and 3 in Streptococcus pneumonia & 6LNW with Epinephrine	PRO 21,TRP 22, ARG 28,GLU 30, ASN 415	29.348	-7.02	-7.13 µM	6

Table 5 — Docking validation of ligands docked with Nontypeable Haemophillus influenzae SapA in complex with heme-responsible for causing COPD

Protein name and pdb id	Amino acid involved in interaction	RMSD value(Å)	Binding energy (kcal/mol)	Inhibition constant	No. of hydrogen bonds
Nontypeable Haemophillus influenzae SapA in complex with heme&7OFW with cubebinol	GLY55, THR62, GLU63, LYS68,TYR166, ALA203	178.420	-8.17	1.03 µM	6
Nontypeable Haemophillus influenzae SapA in complex with heme&7OFW with Amoxicillin	GLY 55, ALA 203, GLN205, GLY461, ASN 462, and GLY 467.	179.842	0.37	14.801 µM	7

The hydrogen bonds in the docked complex determines the close affinity of the macromolecule and ligand, in this complex the no of hydrogen bonds in the docked complex was found to be 5 and that is a quiet good score[31]. The inhibition constant is determined to be inversely propotional for the binding energy and it is expected to be lower in the docked complex and it is 534.89 nM[32]. The commercial drug compound used to cure asthma was refered to be epinephrine, their docking score was predicted with the protein molecule responsible for causing asthma,The standard drug's RMSD value 29.348 Å and the inhibition constant value was found to be 7.13 µM also it possess 6 hydrogen bonds.

In Fig. 2, from image (a) we could clearly view the amino acid molecules like VAL127, ALA17, ARG28, PHE62, HIS58, GLN 56 and THR 20 from the macromolecule of asthma causing streptococcus pneumoniae have a high affinity hydrogen bonds with the ligand molecule cubebinol. And their bond length defines the affinity of the molecule[27]. From Fig. 2b, we could infer the hydrogen bonds made by epinephrine and the macromolecule as PRO 21, TRP 22, ARG 28, GLU 30, and ASN 415.

The ligand compound cubebinol was docked with the protein which is responsible for causing COPD and from Table 5, we could see the active site predicted amino acid molecules involved in the interaction and they are GLY55, THR62, GLU63, LYS68, TYR166, and ALA203. The docked complex possessed a good binding affinity value of -8.17 kcal/mol whereas the binding affinity of the docked complex with ligand as the standard drug was found to be very poor with 0.37 kcal/mol[29]. The cubebinol and macromolecule complex had the lower RMSD value of 178.420 Å Along with the lower inhibition constant 1.03 µM, the macromolecule and the cubebinol complex are bonded with 6 hydrogen bonds while the docked complex with the commercially used drug to treat COPD was referred to be as amoxicillin and it showed a very poor binding affinity, with higher RMSD value of 179.842 Å along with inhibition constant 14.801 µM and the amoxicillin and the macromolecule complex holds 7 hydrogen bonds in it.

From Fig. 3a, we could infer the complete docked complex of nontypeable Haemophillus influenzae SapA in complex with heme along with the ligand molecule cubebinol. Here, we could clearly have a view of bonding length of the complex. From Fig. 3b we could clearly infer the binding sites of the ligand compound amoxicillin with the macromolecule and

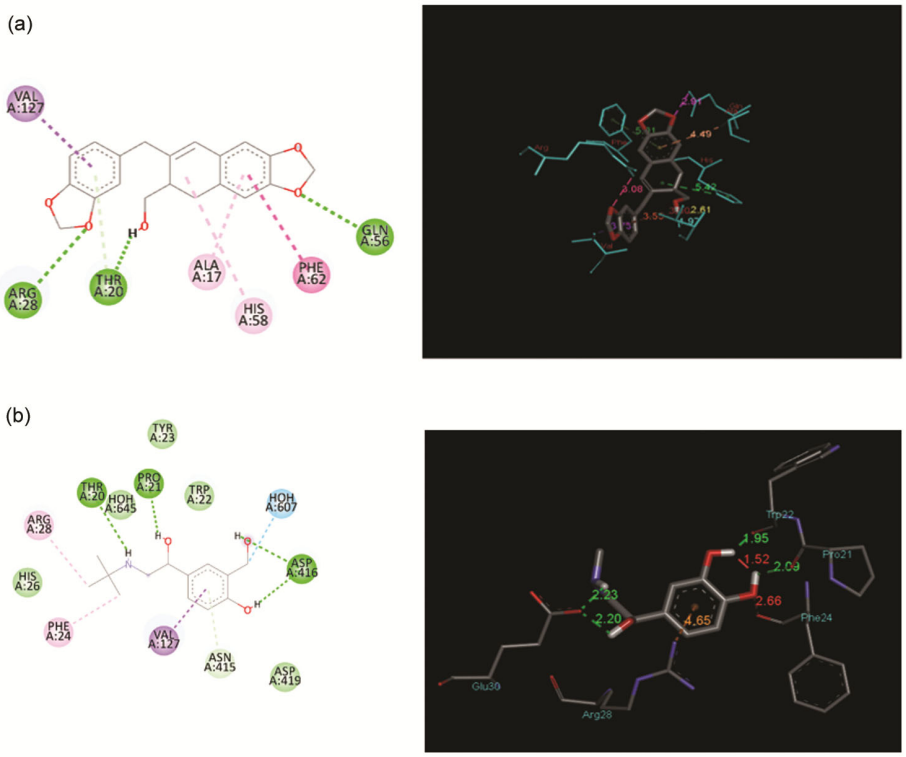


Fig. 2 — Two (left) and three (right) dimensional images of the crystal structure of accessory secretory protein 1, 2 and 3 in Streptococcus pneumoniae along with the ligand (a) cubebinol and (b) epinephrine

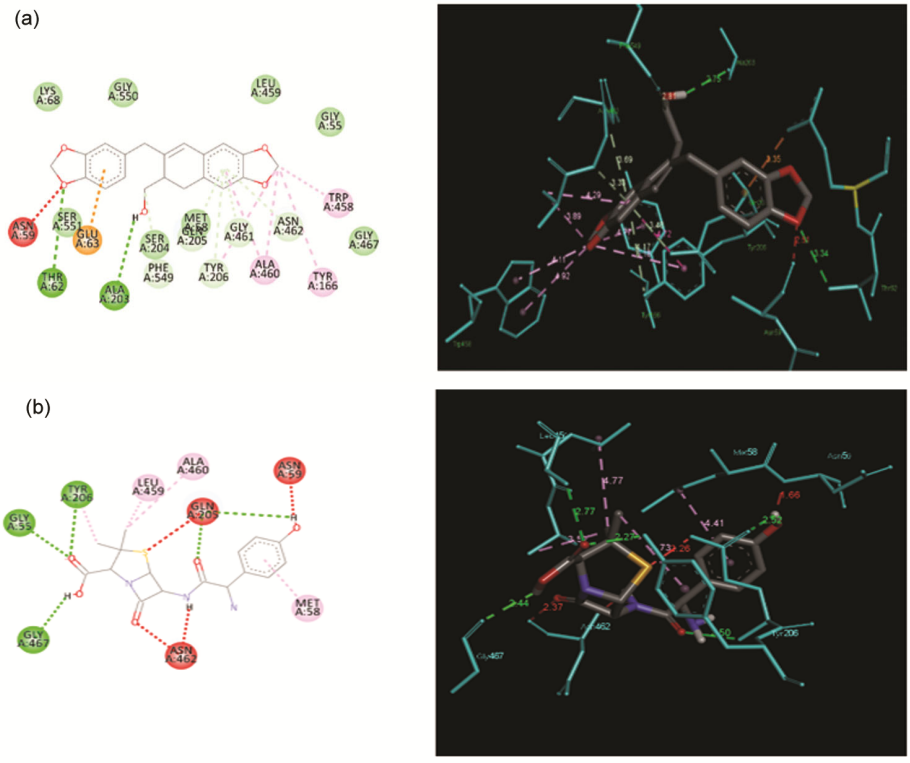


Fig. 3 — Two (left) and three (right) dimensional image of Nontypeable Haemophillus influenzae SapA in complex with heme along with the ligand molecule (a) cubebinol and (b) amoxicillin

the sites are GLY 55, ALA 203, GLN205, GLY461, ASN 462, and GLY 467.

The ligand compound cubebinol was docked with the protein which is responsible for causing cystic fibrosis from Table 6, we could see the amino acids which are all involved in the binding are ILE10, ASP15 with the binding energy of -5.48 kcal/mol whereas the commercial drug used to cure cystic fibrosis was referred to be sulfamethoxazole which showed a poor binding affinity value than cubebinol and the binding energy value of the standard drug complex was found to be -4.92 kcal/mol[29]. Cubebinol and the COPD macromolecule had low RMSD score of 6.848 Å and their inhibition constant was 96.65 µM with 2 hydrogen bonds.The docked complex of sulfamethoxazole with the macromolecule showed RMSD score of 6.264 Å with inhibition constant value 61.29 µM and it contains only the hydrophobic interaction with no hydrogen bonds

From Fig. 4a, we could infer the docked complex of cubebinol with its binding site in the determined active site region of the macromolecule and their bond

Table 6 — Docking validation of ligands docked with cystic fibrosis transmembrane conductance regulator: solution structures of peptides based on the phe508 region, the most common site of disease-causing delta-f508 mutation-responsible for causing cystic fibrosis

Protein name and pdb id	Amino acid involved in interaction	RMSD value(Å)	Binding energy (kcal/mol)	Inhibition constant	No. of hydrogen bonds
Cystic fibrosis transmembrane conductance regulator: solution structures of peptides based on the phe508 region, the most common site of disease-causing delta-f508 mutation & 1CKW with cubebinol	ILE10, ASP15	6.848	-5.48	96.65 µM	2
Cystic fibrosis transmembrane conductance regulator: solution structures of peptides based on the phe508 region, the most common site of disease-causing delta-f508 mutation & 1CKW with sulfamethoxazole	ILE 10, and TYR 17	6.262	-4.92	61.29 µM	3

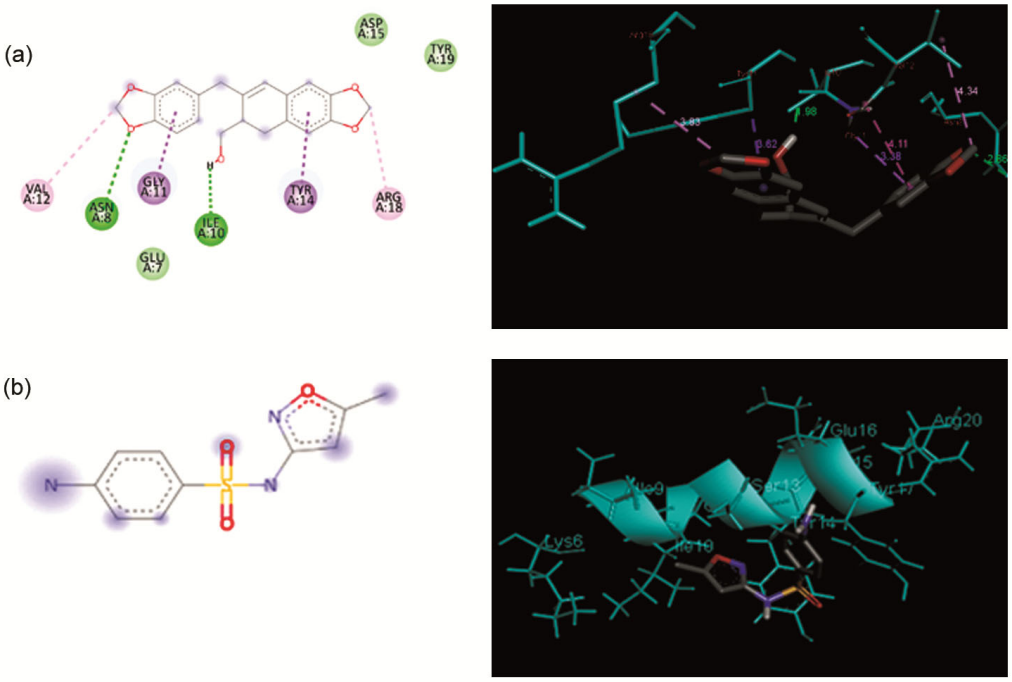


Fig. 4 — Two (left) and three (right) dimensional image of Cystic fibrosis transmembrane conductance regulator: solution structures of peptides based on the phe508 region, the most common site of disease-causing delta-f508 mutation along with the ligand molecule (a) cubebinol and (b) sulfamethoxazole

lengths were studied. From Fig. 4b we could infer the docked complex with sulfamethoxazole, its binding site in the determined active site region of the macromolecule and their bond lengths were studied, their pocket atoms were determined to be ILE 10, and TYR 17.

The ligand compound cubebinol was docked with the protein which is responsible for causing Lung cancer. From Table 7, we could see the amino acids involved in the interaction were 120B PRO, 169B ASN with the binding energy of -4.82 kcal/mol whereas the standard drug used to cure lung cancer was referred to be Etoposide, the complex of Etoposide and the macromolecule showed poor binding affinity value of 5.62 kcal/mol[29]. The cubebinol and macromolecule docked complex possessed low RMSD value of 22.594 Å and its inhibition constant was found to be 291.60 µM with 2 hydrogen bonds, and the macromolecule and etoposide complex possessed RMSD value of 22.99 Å and its inhibition constant was found to be 3.10 mM with 7 hydrogen bonds.

From Fig. 5a, we could examine the docked complex of Bcl_2-Navitoclax (ABT-263) Complex with the ligand molecule cubebinol. In which the docked complex nature could be clearly seen with the bond distance values. From 5b, we could examine the docked complex of Bcl_2-Navitoclax (ABT-263) complex along with the ligand molecule Etoposide, in which the docked complex nature could be clearly seen with the bond distance values with the amino acid pockets like HIS 117, THR 119, THR 122, ARG 161 and GLU 162.

Table 7 — Docking validation of cubebinol docked with Bcl_2-Navitoclax (ABT-263) Complex-responsible for causing lung cancer

Protein name and pdb id	Amino acid involved in interaction	RMSD value(Å)	Binding energy (kcal/mol)	Inhibition constant	No.of hydrogen bonds
Bcl_2-Navitoclax (ABT-263) Complex&4LVT with cubebinol	120B PRO,169B ASN	22.594	-4.82	291.60 µM	2
Bcl_2-Navitoclax (ABT-263) Complex&4LVT with Etoposide	HIS 117, THR 119, THR 122, ARG 161 and GLU 162.	22.99	5.62	3.10 µM	7

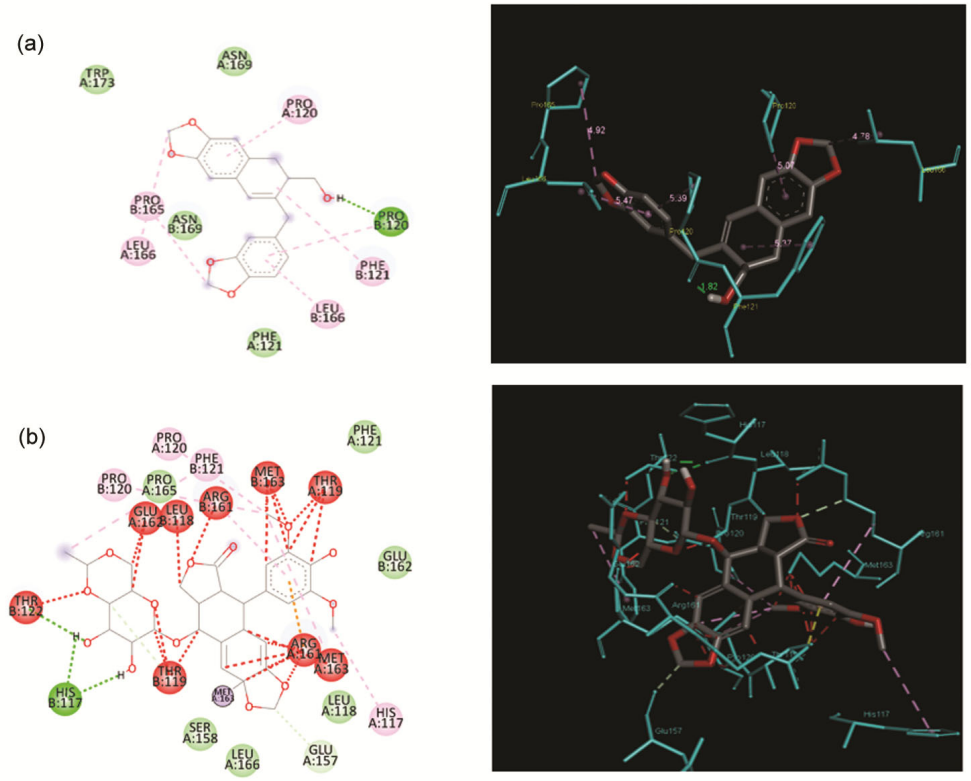


Fig. 5 — Two (left) and three (right) dimensional image of Bcl_2-Navitoclax (ABT-263) Complex along with the ligand molecule (a) cubebinol and (b) etoposide

The ligand compound cubebinol was docked with the protein which is responsible for causing tuberculosis. From Table 8, we could infer that the docked complex shows the good binding energy of -8.45 kcal/mol whereas the standard drug used to treat tuberculosis was referred to be as Isoniazid[33] and the binding affinity of the docked complex of macromolecule and the standard drug Isoniazid was found to be -7.02 kcal/mol which is good docking score but it is lower value when compared with the docking score of the cubebinol and the macromolecule complex[29]. The cubebinol and the macromolecule responsible for causing tuberculosis showed low RMSD value of 38.485 Å and it was bonded with 3 hydrogen bonds and their inhibition constant value was determined to be 64182 nM. The macromolecule complexed with Isoniazid showed the RMSD value of 42.409 Å with the inhibition constant value of 7.14 µM and complex possessed only the hydrophobic interactions and not the hydrogen bond interactions.

Figs 6a and 6b show the docked complex confirmations along with the bond distance between the cubebinol macromolecule complex and the Isoniazid and macromolecule complexes, respectively. Thus, the docking validations shows the values of docking score of the macromolecules along with the standard drug compounds and cubebinol. The docking with the phytocomponent cubebinol is

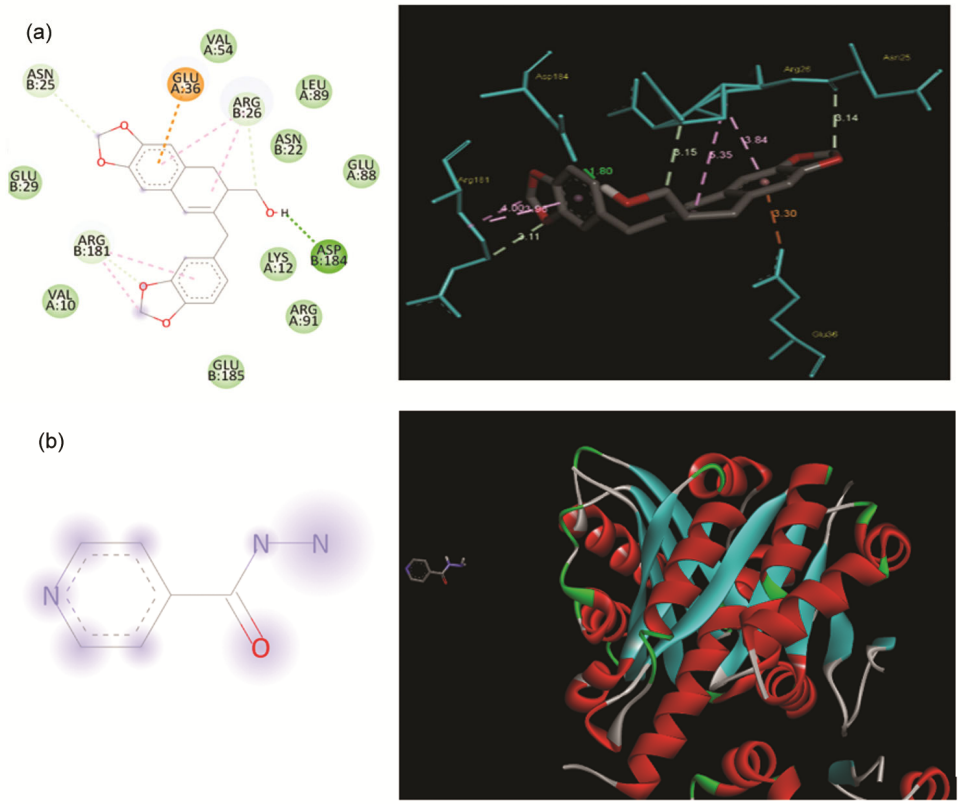


Fig. 6 — Two (left) and three (right) dimensional image of Mycobacterium tuberculosis FtsZ in complex with GDP along with the ligand (a) cubebinol and (b) Isoniazid

Table 8 — Docking validation of ligands docked with Mycobacterium tuberculosis FtsZ in complex with GDP-responsible for causing tuberculosis

Protein name and pdb id	Amino acid involved in interaction	RMSD value (Å)	Binding energy (kcal/mol)	Inhibition constant	No. of hydrogen bonds
Mycobacterium tuberculosis FtsZ in complex with GDP & 6YM1	26B ARG,184B ASP, 185B GLU	38.485	-8.45	641.82 nM	3
Mycobacterium tuberculosis FtsZ in complex with GDP &6YM1–isoniazid	No hydrogen bonds involved	42.408	-7.02	7.17 µM	0

proven to be efficient with the lower binding affinity value.

Pharmacokinetic property and Lipinski rule of 5 of cubebinol

The Lipinski rule of 5 was tested for the ligand compound where the ligand does not contain more than 5 hydrogen bonds, no more than 10 hydrogen bond acceptors, the molecular mass of the compound does not exceed 500 Dalton and Clog P value does not exceed 5. Thus the ligand compound cubebinol passed the Lipinski rule of 5 test which was tested using the swissADME tool[34]. The ligand compound also passed the Ghose rule thus states that the molecular weight must be within 160-480, molecular refractivity must be within 40 and130, total number of atoms count must be 20-70 and the calculated log p value must be within -0.4 to 5.6. Similarly the ligand passed the Veber and Egan rules[35]. The Egan value states that the bioavailability score must be in the limit of $0 \geq$ tPSA ≤ 132 Å2 and $-1 \geq \log P \leq 6$[36]. Thus with these drug likeliness properties, the evaluation of the ligand compound shows that cubebinol exhibits a good drug likeliness nature as shown in Table 9.

The ADMET property could be described as the pharmacokinetic property of a drug. In simpler words it is what body does to the drug. The ADMET properties play a vital role in designing and discovery of drug molecules. The ADMET properties of the compounds help us to carry out and validate the environmental and human hazard assessment. The usual role of a drug is that it must reach the site of action directly and after the completion of the process it must be completely excreted from the body without causing any toxic effects in the body. Hence it is one of the mandate steps in drug discovery to analyze the ADMET property. Absorption is considered as the initial step, in which the drug get adsorbed through oral or intravenous route and enters into the blood stream[37]. Distribution describes the concentration of the drug in the tissues or organs, which could be evaluated by *in vivo* and preclinical process. Bioavailability is defined as the ability of the drug to be adsorbed and utilized by the body, once the drug enters the systemic circulation its bioavailability could be analyzed. Metabolism is an enzymatic process in which the drug gets converted into hydrophilic metabolites before and/or after executing its action. Subsequently the drug could be eliminated via excretion process. Excretion is defined as the irreversible loss of the drug metabolites from the body, hence they were evaluated in study through computational mode[38].

Table 9 — Druglikeliness property of the ligand compound (cubebinol)

Property name	Property value
Number of Lipinski's rule of 5 violations	0
Lipinski's rule of 5	Passed
Number of Ghose rule violations	0
Ghose rule	Passed
Veber rule	Good
Egan rule	Good
GSK 4/400 rule	Good
Weighted quantitative estimate of drug-likeness (QEDw) score	0.93

Table 10 — ADMET properties of cubebinol

Property name	Property value
Bioavailability score	0.55
Solubility class [ESOL]	Moderately soluble
Solubility class [Silicos-IT]	Moderately soluble
Blood Brain Barrier permeation	Yes
Gastrointestinal absorption	High
Log K_p (Skin permeation, cm/s)	-6.08
Number of PAINS structural alerts	0.0
Number of Brenk structural alerts	0.0
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
P-glycoprotein substrate	Yes

Table 10 shows the absorption, distribution, metabolism, excretion and toxicity profile of the ligand compound. The main part of the ADMET property is the bioavailability score and the compound had 0.55 as its value, it also states that if a compound passes the Lipinski rule of 5 then it is proposed to have a good bioavailability score[36]. The gastro intestinal absorption is one of the vital one which would determine the bioavailability of the compound, the compound said to produce a higher value of GI absorption thus it shows that it is efficient.

In the present investigation, Swiss ADMET software was involved to check the toxicity of cubebinol. As per our present findings (Table 10), we found that cubebinol possess a good ADMET properties and drug availability score, therefore, we infer that the compound is nontoxic and could be regarded as safe for consumption[39]

Conclusion

Till date our world completely suffers due to several different forms of respiratory ailments, the mortality rate in the respiratory disease also seems to be one of the alarming threats in the current situation.

Thus one of the efficient pharmacokinetic properties providing phytocomponent was considered and its potency was evaluated against the major 5 respiratory diseases which does not have a complete cure till date. As the initial step of evaluation the computational investigation of the macromolecules and ligand compound was performed and as a result the compound bound well with the active sites of the protein molecule with higher binding affinity. The higher binding affinity was proposed with the protein molecules which is responsible for causing asthma with the higher binding affinity of -8.56 kcal/mol. The binding affinity for the tuberculosis causing protein was estimated to be -8.45 kcal/mol, for the COPD causing protein the binding affinity was found to be -8.17 kcal/mol, then for cystic fibrosis causing protein the binding affinity seemed to be -5.48 kcal/mol and for the protein responsible for causing the lung cancer gave the binding affinity of -4.82 kcal/mol. All the currently usable commercial drug molecules possessed a huge difference by providing very poor binding affinities with the ailment causing bacterial macromolecules. Thus by both the pharmacokinetic test as well as the docking validation we could evaluate that the docked ligand compound cubebinol is potent drug against several fatal bacterial respiratory disease.

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