Available online on 15.06.2021 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Targeting β-glucan synthase for Mucormycosis "The 'black fungus" maiming Covid patients in India: computational insights

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Article Info:

Article History:

Received 17 April 2021
Review Completed 21 May 2021
Accepted 27 May 2021
Available online 15 June 2021

Cite this article as:

Sharma AD, Kaur I, Targeting β-glucan synthase for Mucormycosis "The 'black fungus" maiming Covid patients in India: computational insights, Journal of Drug Delivery and Therapeutics. 2021; 11(3-S):9-14
DOI: http://dx.doi.org/10.22270/jddt.v11i3-S.4873

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Abstract

Black fungus also known as Mucormycosis, has recently devastated some states of India. It has been declared pandemic now. Inhibitors of glucon synthesis pathways have been evaluated to curtail the Mucormycosis but still at infancy stage. Due to key role in glucon synthesis, in the present study β -glucan synthase has been regarded as a suitable target for drug design. *In-silico* docking and pharmacological study was designed to evaluate the effect of potent bioactive molecule 1-8 cineole present in essential oils of eucalyptus plant leaves against β -glucan synthase enzyme. Till date there is no work is undertaken on *in-silico* analysis of this compound against β -glucan synthase. Patch-dock analysis was used for docking. Ligand Protein 2D and 3D Interactions were also studied. Drug likes and toxicity profile was also evaluated. Cancer cell line toxicity profile was also studied. The calculated parameters such as docking score indicated effective binding of 1-8 cineole to β -glucan synthase -protein. Interactions results indicated that, β -glucan synthase enzyme and 1-8 cineole complexes forms hydrogen and hydrophobic interactions. 1-8 cineole also depicted sufficient level of cancer cell line toxicity. Drug likeliness profiles by assaying absorption, distribution, metabolism, excretion and toxicity (ADMET) studies provided guidelines and mechanistic scope for identification of 1-8 cineole as potent anti-fungal drug. Therefore, essential oil from eucalyptus may represent potential herbal treatment to act as anti-fungal drug.

Keywords: COVID-19, black fungus, Eucalyptus oil, Herbal Drug

INTRODUCTION

At present world is experiencing bad situation due to COVID-19 a deadly pandemic disease. Yet it is not over, an another disease grim the nation being called as " black fungus" also known as "mucormycosis". In tandem with conversations around COVID-19, the black fungal infection has also intrigued people. In some of the states like Maharashtra and Gujarat and particularly in Rajasthan, this disease has been declared as pandemic. Doctors observed that this fungus infected only those individuals who are in severely immune-compromised health conditions, such as COVID-19 patients having diabetes or high uncontrolled sugar levels after recovery[1]. Black fungus is a rare fungal infection that affects 1 in 10,00,000 individuals, but with an overall mortality rate of 50 per cent[1]. It was observed that indiscriminate use of steroids for the treatment of COVID-19 patients is the possible reason for this infection. It was cited that when the body's immune system fights against virus, use of steroids in COVID-19 patients reduced inflammation in the lungs but uncontrolled use of steroids doses also reduce immunity and raise blood glucose level due to less physical activity in diabetic and non-diabetic people thus increased the risk of catching the black fungal infection. Black fungal infection affects the sinuses, lungs, the brain and can be life-frightening in severely immune-compromised people. The most observed typically symptoms are like: Blackish discolouration around the nose, bleeding and stuffy nose; Black crusts in the nose, loosening of teeth, jaw involvement, one-sided facial pain or numbness, and swelling in eyes; drooping of eyelids; pleural effusion, worsening of respiratory symptoms and blurred loss of vision[1].

Zavrel and White (2020)[2] demonstrated that inhibition of β-1,3-glucan biosynthesis by using inhibitor drugs like amphotericin/echinocandins inhibited fungal growth thus abolished replication. Due to clinical limitations of fungicidal agents, high price, inevitable toxicities, and the appearance of drug resistance, the synthesis of effective and safe fungicidal agents based on novel antifungal targets is instantly needed[3]. The key drug molecules which act as inhibitors of β-1,3-glucan biosynthesis have been advocated as potential therapeutic agents for treating fungal infections[4]. It is a glucosyl-transferase enzyme involved in the generation of beta-glucan in fungi which is key component of cell wall construction. Earlier study cited that 1,3-beta-glucan synthase is a potential target to design anti-fungal drugs as no such structure exits in humans[5-6]. We present here our perspective on the potential use of a bioactive compound 1-8 cineole as a potential treatment modality for black fungus by targeting fungal enzyme 1,3-beta-glucan synthase.

Essential oil from eucalyptus species contains a number of oxygenated and non-oxygenated bio- actives. The value of Eucalyptus oil for medicinal purposes is based largely on the 1,8-cineole (cineole or eucalyptol) content of oil which is a

major component (about 90%) of eucalyptus oil in all *Eucalyptus* plants[7]. It is an oxygenated-monoterpenoid and cyclic ether and due to this bioactive, eucalyptus oil has been empirically used as antimicrobial agents, but little is known about its antifungal potential[8-9]. Some earlier studies have reported the antifungal propriety of hot water extracts from dry leaves against dermatophytes, filamentous and Candida albicans[10-11] . We hypothesize that due to richness of bioactive like 1,8 cineole from Eucalyptus essential oil, it has the capability to prevent infection of black fungus. Therefore the research objective of the current study was *in-silico* analysis and molecular docking studies pertain to 1,8 cineole in relation with 1,3-beta-glucan synthase . Thus the outcomes of the current study would provide researchers with prospects to recognize the accurate fungicidal drugs during COVID19 medications.

MATERIALS AND METHODS

Ligand modelling

The instinctive ligands for 1,3-beta-glucan synthase protein structure was 1,8 cineole . SMILES (simplified molecular-input line-entry system) was retrieved for 1,8 cineole and converted to their corresponding 3D structures by using UCSF-chimera and saved in .pdb format.

Protein receptor preparation and Molecular Docking

X ray crystal structures of 1,3-beta-glucan synthase (PDB ID: 4m80) was retrieved from PDB web cite (https://www.rcsb.org/). The target enzymes were cleaned and prepared before docking study. Before the docking studies, the protein structure was first prepared using the dock prep set up in chimera software. The dock preparation is an optimization part that corrects atomic and bond length, structure, charges anomalies. Original inhibitors and water molecules were detached from the 1,3-beta-glucan synthase enzyme structure and any missing hydrogen atoms were added. PatchDock tool was used for docking study of the 1-8 cineole over 1,3-beta-glucan synthase enzyme (https://bioinfo3d.cs.tau.ac.il/PatchDock/). For this ligand (1-8 cineole) and receptor molecule in .pdb file formats were uploaded to PatchDock server and job was executed. The best generated docked structure was downloaded and saved as .pdb file. The docked complex structure output formats were submitted into Biovia Discovery Studio Visualizer 2020 and Chimera tools in order to study 3D conformations, surface analysis and to map the interaction of the resulting docked complexes (https://projects.biotec.tu-dresden.de/plip-web/plip/index).

Binding Mode of Docked Complexes

Plip tool was use to find out residues involved in 3-D interactions (https://projects.biotec.tu-dresden.de/plip-web/plip/index/). 2-D interactions were also calculated using discovery studio 2020 client software.

Drug-likeness and toxicity

1,8 cineole was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) with PubChem CID 16142. Lipinski's rule of five was used to calculate drug like properties. SWISSADME prediction tool was used to find Lipinski's rule of five (http://www.swissadme.ch/ http://lmmd.ecust.edu.cn/admetsar1/predict/) and to find out ADMET (Absorption, Metabolism, Toxicity and Excretion) properties. Bioactivity analysis was carried out using molinspiration tool (https://www.molinspiration.com/cgi-bin/properties). Bioactivity was based on following score: if the bioactivity score is (>0), then it is active, if (-5.0-0.0) then moderately active, if (< -5.0) then inactive.

Active sites prediction in 3D modeled receptor

CASTp (The Computed Atlas of Surface Topography of proteins) web tool was used to predict active sites residues in the 1,3-beta-glucan synthase protein. CASTp is an online tool used in identification and dimension of cavities on 3D protein structures. Default value of 1.4 Angstroms was used as probe radius.

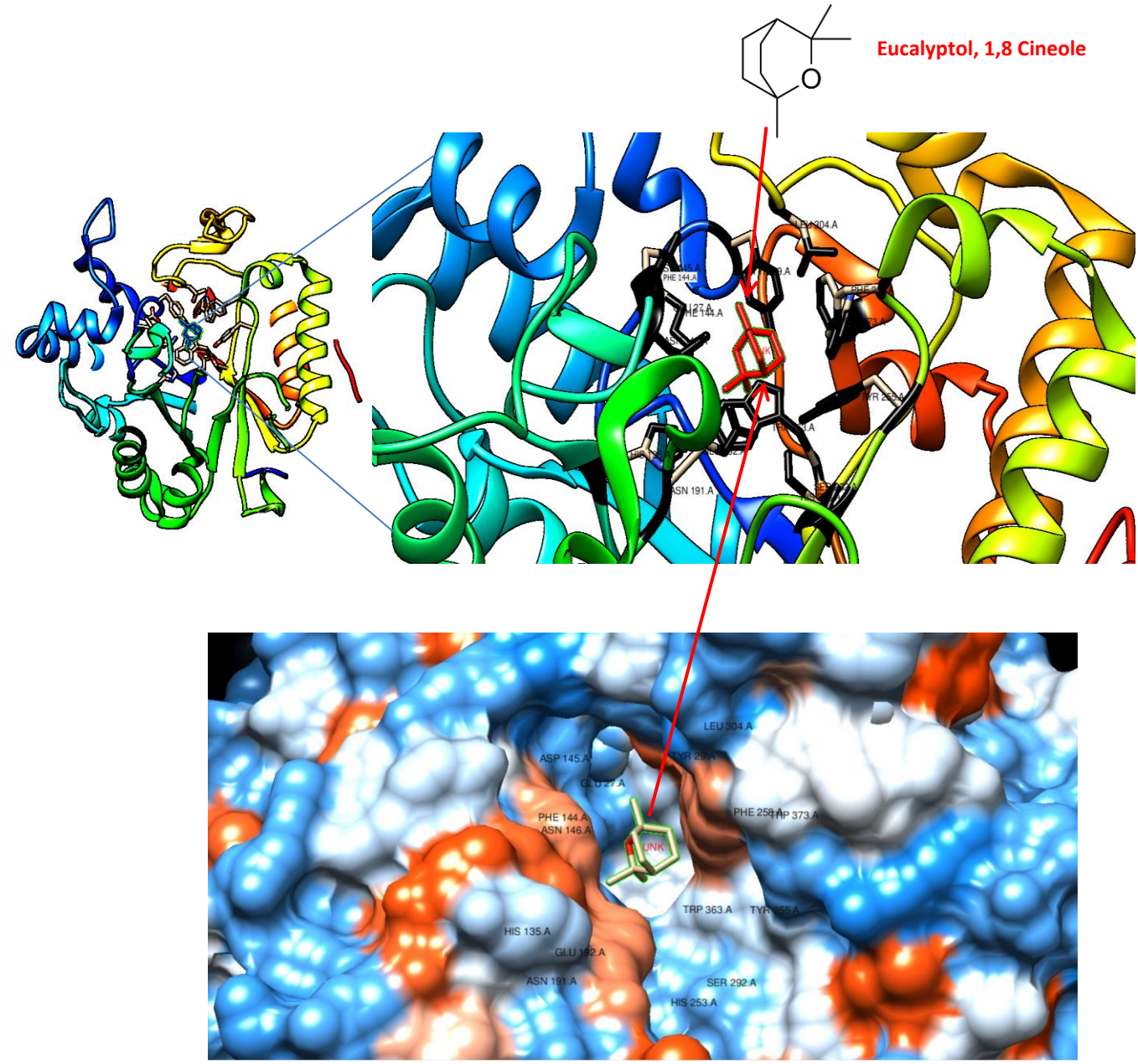
Cell line toxicity prediction

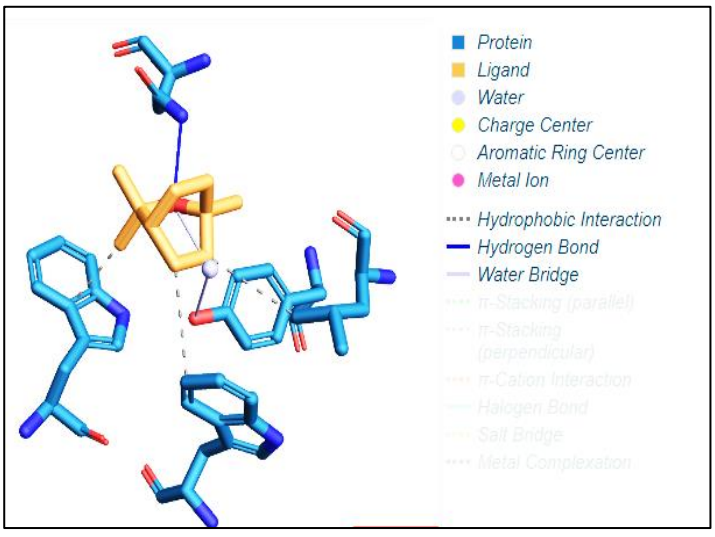
Cytotoxic effect of 1,8 cineole was determined using CLC-Pred (Cell Line Cytotoxicity Predictor) which is a web-service for in-silico prediction of chemical compounds in cancer and non-transformed cell lines based on structural formula. CLC-Pred (http://way2drug.com/Cell-line/) offers a prediction of the cytotoxicity of a chemical bio active compound to assess the significance of the compounds inclusion in experimental screening.

RESULTS AND DISCUSSION

Recent observations have documented that individuals who are in severely immune-compromised health conditions after COVID-19 having diabetes or high uncontrolled sugar infected with a disease caused by "black fungus". It was also suggested that antifungal agents can be used as inhibitors of fungal infections by targeting 1,3-beta-glucan synthase as no such structure exits in humans[5-6]. Hence, targeting glucon metabolism may offer new active antifungal approach to treat black fungus. So the objective of the present study was to evaluate therapeutic potential of 1-8 cineole against 1,3-beta-glucan synthase, a major glycolytic enzyme for synthesizing glucon in fungal cell wall.

Molecular docking using patchDock docking tool that was used to find out interactions of inhibitor 1-8 cineole with 1,3-beta-glucan synthase protein revealed 20 diverse poses based on the dock score and area. Model depicted maximum score values and maximum binding affinity is shown in Table 1. Docking pose and molecular interactions of 1-8 cineole with 1,3-beta-glucan synthase are shown in Figure 1. It was observed that 1-8 cineole successfully docked in the active site of with 1,3-beta-glucan synthase enzyme with dock score 3094. Fungal β-glucan synthase is composed of at least two subunits: a putative C-terminal catalytic subunit and an N-terminal regulatory subunit[13]. From *in-silico* analysis it was found that 1-8 cineole successfully docked on catalytic C-terminal domain. According to the Plip server and Biovia studio results, the interaction of 1-8 cineole in C-terminal domain of β-glucan synthase was mediated by both hydrophobic and hydrogen bond interactions. Hydrophobic interactions were observed via LEU 304, TRP 363, 373 at atomic distances of 3.98, 3.21, and 3.84 Å, respectively (Figure 1, Table 2). Hydrogen bond interactions of 1-8 cineole were also observed via ASN 146 at atomic distances of 2.50. Notably, one water bridge was also observed with TYR 29. Active site prediction by CAST-P server also revealed the presence of interacting residues in the major cavity of β-glucan synthase protein (Table 3). With CASTp active site prediction, a major pocket was identified with Area (SA) of 4364 and Volume (SA) of 6092. Since 1-8 cineole poses high affinity towards β-glucan synthase enzyme, so it absolutely was postulated that β-glucan synthase becomes closed upon binding with 1-8 cineole that in turn induces conformational change in β-glucan synthase and stop further execution of catalytic action hence down regulate the infectivity and spread of black fungus into the host cell.





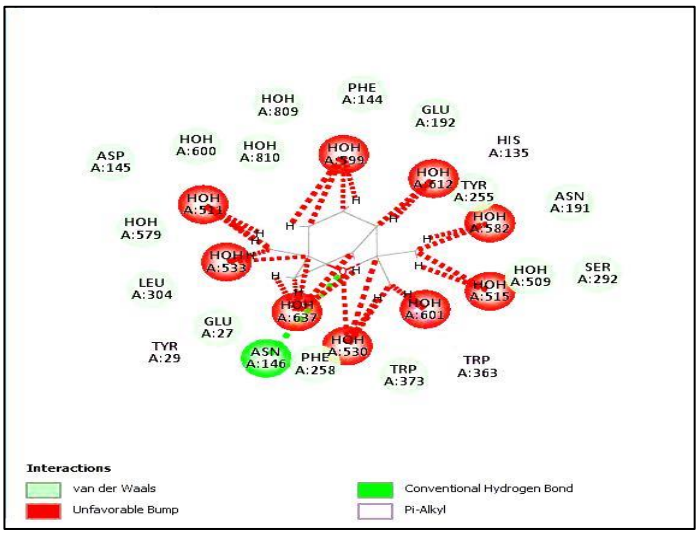


Figure 1: Docked complex and molecular interactions of 1,3-beta-glucan synthase with 1-8 cineole

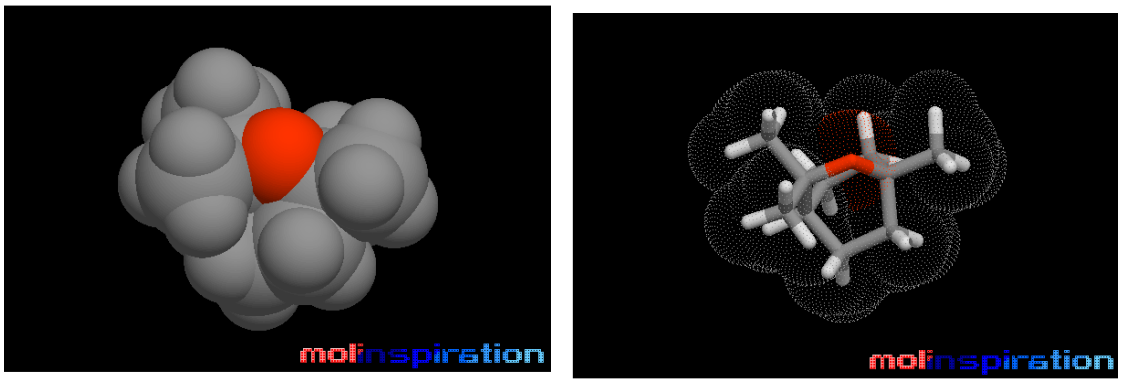


Figure 2: Molecular lipophilicity potential (MLP)/ polar surface area (PSA) views of 1-8 cineole. Hydrophobic areas: encoded by violet; Hydrophilic areas: red.

Table 1: Docking analysis of 1-8 cineole molecules with 1,3-beta-glucan synthase protein

Solution No	Score	Area	ACE	Transformation
1-8 cineole	3094	333.30	-84.12	0.13 -0.33 -1.94 -3.36 14.06 3.04

Interacting residues and length (4 A°)			Binding pocket residues within 4 A° radius
H-bond interactions	**Water bridge**	**Hydrophobic interactions**	
ASN146	TYR29	LEU304, TRP363,373	LEU 304, PHE 144, ASN146, HIS 135, GLU 192, ASN 19, TRP 363, 373, TYR 255, SER 292, HIS 253, SR 145, GLU 27, PHE 144, ASN 146, TYR 29

Table 2: 3-D intercations of 1,8 cineole with 1,3-beta-glucan synthase

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	304A	LEU	3.98	3559	2438
2	363A	TRP	3.21	3562	2923
3	373A	TRP	3.84	3557	3016

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	146A	ASN	2.50	2.97	109.17			1153 [Nam]	3553 [O3]

Water Bridges

Index	Residue	AA	Dist. A-W	Dist. D-W	Donor Angle	Water Angle	Protein donor?	Donor Atom	Acceptor Atom	Water Atom
1	29A	TYR	3.05	3.64	104.83	115.04		187 [O3]	3553 [O3]	3378

Pharmacokinetic analysis of 1-8 cineole using ADMET properties was studied. 1-8 cineole scanning results are illustrated in Table 4. Topological polar surface area (TPSA) value was 9.23°A squared. It indicated good permeability of 1-8 cineole cell membranes to enter Blood Brain Barrier (BBB)[14]. Pharmacokinetics parameters and ADMET factors are key parameters for success of mostly drugs during clinical trials. Log Po/w (lipophilicity indicator, octanol-water partition coefficient) was 2.67, indicating 1-8 cineole was optimal BBB penetration. GI (Gastrointestinal tract absorption) of 1-8 cineole was high (Table 4). In order to exert a toxic effect, drug molecules have to be absorbed from intestinal tract in the body. Further, 1-8 cineole shown non inhibitory activity against cytochrome P series of enzymes, involved in liver detoxificatyion of toxins from body.

3D molecular structures showing Molecular Lipophilicity Potential (MLP) and Polar Surface Area (PSA) are also shown in Fig. 2. MLP is convenient property to rationalize numerous molecular ADME characteristics (for example: plasma-protein binding or membrane penetration). Bioactivity of 1-8 cineole as the drug was calculated online by using Molinspiration drug-likeness score. Table 3 depicts Bioactivity score of 1-8 cineole. Ion channel property of 1-8 cineole was high(>0) while enzyme inhibitor and protein inhibitor activities were high moderate (<0).

Cell line toxicity results revealed that bio active 1-8 cineole was toxic to tumor cell lines (Table 5. It was found that Pa > Pi , indicated that studied compound was belonged to the sub-class of active compounds i.e it resembles the structures of molecules, which are the most typical in a sub-set of "actives" in PASS training set) as per Way2Drug server prediction.

Table 3: Protein target structure, native ligand and active site amino acids

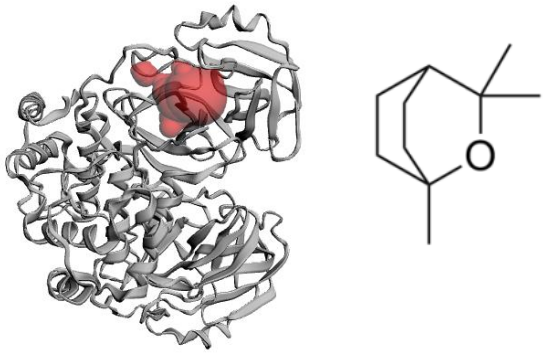
4m80		LEU 304, PHE 144, ASN146, HIS 135, GLU 192, ASN 19, TRP 363, 373, TYR 255, SER 292, HIS 253, SR 145, GLU 27, PHE 144, ASN 146, TYR 29	4364.455	6092.808

Table 4: ADMET properties of ligands

Lipinski's rule of five					**ADMET properties**							
Molecular weight	**Num. H-bond acceptors**	**Num. H-bond donors**	**Molar refractivity**	**lipophilicity logP**	**GI absorption**	**Consensus Log Po/w**	**TPSA**	**BBB permeant**	**P-gp substrate**	**CYP 3A4 inhibitor**	**Log Kp (skin permeation)**	**Log S (ESOL)**
154.25 g/mol	1	0	47.12	2.58	High	2.67	9.23 Å^2	Yes	No	No	-5.30 cm/s	-2.52

Molinspiration bioactivity	**score**
	1-8 cineole
GPCR ligand	-0.93
Ion channel modulator	0.01
Kinase inhibitor	-1.60
Nuclear receptor ligand	-1.07
Protease inhibitor	-0.90
Enzyme inhibitor	-0.1

Table 5: Cancer cell line prediction result

Pa	Pi	Cell line	Cell line Full name	Tissue	Tumor type
0.949	0.002	NCI-H187	Small cell lung carcinoma	Lung	Carcinoma
0.770	0.002	Raji	B-lymphoblastic cells	Haematopoietic and lymphoid tissue	Leukemia
0.817	0.003	BXPC-3	Pancreatic adenocarcinoma	Pancreas	Adenocarcinoma
0.925	0.003	LoVo	Colon adenocarcinoma	Colon	Adenocarcinoma
0.908	0.005	A549	Lung carcinoma	Lung	Carcinoma
0.701	0.005	HCT-15	Colon adenocarcinoma	Colon	Adenocarcinoma
0.750	0.005	HepG2	Hepatoblastoma	Liver	Hepatoblastoma
0.719	0.007	PC-3	Prostate carcinoma	Prostate	Carcinoma
0.511	0.011	A2058	Melanoma	Skin	Melanoma
0.605	0.032	MCF7	Breast carcinoma	Breast	Carcinoma

Pa: (probability "to be active"), Pi: (probability "to be inactive")

CONCLUSION

Black fungus has emerged as pandemic in India. Present study revealed molecular docking of 1-8 cineole from eucalyptus essential oil plant against β-glucan synthase protein. However, more *in vivo* and *in vitro* model based studies may pave way these compounds in drug discovery.

Conflict of Interest

No

Sponsorship Information

No

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