Targeting UDP-Glycosyltransferase, Glucosamine-6-Phosphate Synthase and Chitin Synthase by Using Bioactive 1,8 Cineole for “Aspergillosis” Fungal Disease Mutilating COVID-19 Patients: Insights from Molecular Docking, Pharmacokinetics and In-vitro Studies

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Abstract
SARS-CoV-2 (COVID-19)-associated co-infections like “Aspergillosis”, has recently baffled the world. Due to its key role in cell wall synthesis, in the present study UDP-glycosyltransferase, glucosamine-6-phosphate synthase and chitin synthase have been chosen as appropriate targets for molecular docking. The objective of the present study was molecular docking of eucalyptus essential oil component 1,8 cineole against cell wall enzymes followed by in vitro validation. For molecular docking, patch-dock web based online tool was used. Ligand–Protein 2D and 3D Interactions were also studied. Drug likeliness, toxicity profile and cancer cell line toxicity were also studied. Molecular docking results indicated that 1,8 cineole form hydrogen bonding and hydrophobic interactions with UDP-glycosyltransferase, glucosamine-6-phosphate synthase and chitin synthase enzymes. 1,8 cineole also depicted drug likeliness by showing compliance with the LIPINSKY rule, sufficient level of bioactivity and cancer cell line toxicity thus signifying its role as a potent anti-fungal drug.

Keywords Aspergillosis; COVID-19 · Eucalyptus oil · Herbal Drug

1 Introduction

The first case of pneumonia caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) was first reported in China, Wuhan, in December 2019 [1]. Afterwards, this viral disease spread rapidly worldwide causing coronavirus disease (COVID-19) a pandemic. Since the dawn of COVID 19 pandemic, as of August 2020, researchers have documented COVID-19–associated serious co-infections in COVID-19 patients like: aspergillosis [1–3], invasive candidiasis [4], coccidioidomycosis [5], fusariosis [6], mucormycosis [7] and saccharomyosis [8]. Among all, “aspergillosis” contributed to a high mortality rate of up to 67% [9]. Aspergillosis” is a type of infection which is caused by common invasive fungus mold “Aspergillus”, which exists in outdoors and indoors. The rapid rise in fungal infections post 2nd wave of COVID-19 was attributed to the un-regulated use of steroids for COVID patients. It was observed that this fungus affects immune-compromised individuals like COVID-19 patients in recovering state and have diabetes or high un-controlled sugar levels [10]. Doctors observed that uncontrolled use of steroids for COVID patients reduced the body immunity and raised blood glucose level in diabetic and non-diabetic individuals due to poor physical activity which increased the rate of infection of fungus infection [11]. The symptoms of invasive “Aspergillosis” are: running nose, headache, stiffness, chest pain, cough, blood in cough, fever, reduced ability to smell and breathing problems [12].

Due to the rapid emergence of resistant strains of fungus and side effect of antifungal drugs, the synthesis and demand of novel drugs having less toxicity and more effectiveness is instantly required [13]. Hence bioactive molecules that pose properties to act as fungal cell wall-associated enzymes inhibitors have been advocated as key therapeutic drugs to treat fungal infections [14]. Fungal cell wall is a rigid mechanical barrier that plays an key role in protecting fungus
against environmental stresses and other osmotic forces due
to the presence of various structural components like chitin,
glycosyl phosphatidyl inositol anchors (GPI), glucan and
mannoproteins [15]. Therefore, these structural cell wall-
based components represent excellent target sites to design
antifungal drugs. Chitin synthase, UDP-glycosyltransferase
and Glucosamine-6-phosphate synthase are key enzymes
involved in cell wall construction. Earlier studies have paved
the way that these components can serve as an excellent tar-
gets to design antifungal drugs as no such structures exist in
human body [16]. We present here our viewpoint that bioac-
tive molecule eucalyptol also known as 1,8 cineole has the
potential to treat Aspergillus infection by targeting fungal
enzymes such as Chitin synthase, UDP-glycosyltransferase
and Glucosamine-6-phosphate synthase. Chitin synthase is
involved in the process of chitin biosynthesis [16]. UDP-
glycosyltransferase is a key enzyme involved in the first step
in glycosyl phosphatidyl inositol GPI biosynthesis. GPI is a
potential molecule needed for anchoring proteins to the cell
membrane thus involved in the integrity of the fungal cell
wall [17]. Glucosamine-6-phosphate synthase is involved in
the syntheses of N-acetylglucosamine which is an essential
building block for fungal cell wall chitin [18].

Eucalyptus essential oil from eucalyptus species encom-
passes a number of bioactives. Among all, our previous GC-
FID phytochemical based studies revealed that 1,8-cineole (eucalyptol) is a main bioactive of eucalyptus oil in Euca-
lyptus globules [19]. Due to the complex nature of essential
oil, their anti-fungal mechanism of action is still not com-
pletely understood [20]. Previously antifungal potential of
leaf hot water extracts against dermatophytes, filamentous
and Candida albicans has been cited [19–21]. This study
postulated that due to the richness of 1,8 cineole, essential
oil from Eucalyptus globules plants have the potential to
inhibit “Aspergillus”. Hence as an objective this study was
designed to study molecular docking of 1,8 cineole against
Chitin synthase, UDP-glycosyltransferase and Glucosamine-6-
phosphate synthase and wet-lab validation. The present
study outcomes would offer new prospects to identify the
key antifungal drugs during COVID19 medications.

2 Materials and Methods

2.1 Ligand Modelling

1,8 cineole was ligand for Chitin synthase, UDP-glycosyl-
transferase and Glucosamine-6-phosphate synthase struc-
tures. SMILES of 1,8 cineole (CC1(C2CCC(O1)(CC2)C)
C) were retrieved with PubChem CID 2758 from the NCBI-
Pubchem database. UCSF-chimera build structure option
was used to build its 3D structure of 1,8 cineole which was
then saved a pdb file.

2.2 Protein Receptor Preparation and Molecular
Docking

X-ray crystal structures of Chitin synthase, UDP-glyco-
syltransferase and Glucosamine-6-phosphate synthase
with PDB IDs: 4gf8, 5u6m and 1jxa, respectively were
retrieved from PDB web site (https://www.rcsb.org/). The
target enzymes were cleaned from co-crystallized ligand,
selected water molecules and cofactors, prepared energy
minimized before docking study. Before the docking stud-
ies, 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, and charges anomalies. Original inhibitors and
water molecules were detached from the Chitin synthase,
UDP-glycosyltransferase and Glucosamine-6-phosphate
synthase structures and any missing hydrogen atoms were
added. PatchDock tool was used for docking study of the
1,8 cineole over Chitin synthase, UDP-glycosyltransferase
and Glucosamine-6-phosphate synthase enzyme (https://
bioinfo3d.cs.tau.ac.il/PatchDock/). For this both ligand
(1,8 cineole) and receptors molecules in.pdb file formats
were uploaded to the PatchDock server and the job was
executed. The best generated docked structure was down-
loaded and saved as.pdb file. Biovia Discovery Studio
Visualizer 2020, and Plip tool (https://plip-tool.biotec-
tu-dresden.de/plip-web/plip/index) were used to study
docked complexes and their 2D and 3D interactions. For
this, docked complex in pdb format was uploaded and job
was executed with using default parameters.

2.3 Drug-Likeness and Toxicity

To calculate drug-likeness of 1,8 cineole, SMILES
(CC1(C2CCC(O1)(CC2)C)) were used. Various physi-
ociochemical properties, Drug- likeness and pharmacokinetics
studies and ADMET (Absorption, Metabolism, Toxicity
and Excretion) study of 1,8 cineole were conducted using
SWISSADME (http://www.swissadme.ch/ http://Lmmd.
ecust.edu.cn/admetsarl/predict/). For this SMILES of 1,8
 cineole were submitted to SWISSADME server and job
was executed. The toxicity profile was studied by using the
ProTox-II webserver (http://tox.charite.de/protox_II). It
calculates prediction based on different levels of toxicity such
as organ toxicity (hepatotoxicity), oral toxicity, toxicological
endpoints (such as cytotoxicity, carcinotoxicity, mutagen-
icity and immunotoxicity). Web based molinspiration tool
was used to evaluate the bioactivity potential of 1,8 cineole
(https://www.molinspiration.com/cgi-bin/properties). For
toxicity and bioactivity calculation, SMILES of 1,8 cineole
were uploaded to respective servers and jobs were executed.
2.4 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 Chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase proteins. CASTp is an online tool used in the identification and dimension of cavities on 3D protein structures. The default value of 1.4 Angstroms was used as probe radius. For this molecular structures of Chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase proteins were uploaded to CASTp server and the job was executed and output was analyzed.

2.5 Cell Line Toxicity Prediction

In-silico Cancer cell line toxicity analysis of 1,8 cineole was evaluated by using CLC-Pred web-based tool (http://way2drug.com/Cell-line/). For this, input SMILES of 1,8 cineole were submitted to server, the job was executed using the default parameters and output results were analyzed.

2.6 In-vitro Antifungal Activity of Eucalyptus Oil

Two fungal cultures; Aspergillus niger (MTCC 152) and Aspergillus oryzae (MTCC 153) were procured from Institute of Microbial Technology, Chandigarh (India). Strains were maintained on PDA media (Hi-media). Leaves of Eucalyptus globules were collected from campus fields. Eucalyptus oil from leaves of was extracted by Steam-distillation method as described in Sharma and plots. Eucalyptus oil from leaves of was extracted by Steam-distillation method as described in Sharma et al. [19]. Oil obtained was stored in dark bottles at 4 °C till further use. To analyze the antifungal effect of eucalyptus oil on fungal strains, specific concentrations (5–30 µl/ml) of eucalyptus oil were mixed with molten PDA medium followed by manual rotation in Erlenmeyer flask to disperse the oil equally into the medium. 20 ml of molten medium was poured into sterile petri plates (9 cm in diameter). Plates were allowed to solidify at room temperature (~ 35 °C) for 2 h. Mycelial fungal agar discs (6 mm) were taken from the margin of plates having active fungal growth from the 10-day-old pure fungal cultures with the help of sterile cork borer and aseptically inoculated at the middle of the petri plates. Streptomycin (30 mg) was used as a positive control. Control plates were inoculated in the same manner except for essential oil. All plates in triplicate were incubated at 28 °C. After 5 days Inhibition of mycelial growth inhibition was determined by using formula (%) = DC – DT/DC × 100, DC and DT: diameter of control and test colony.

3 Results and Discussion

Early observations cited that COVID patients who are in immune-compromised condition or having uncontrolled diabetes are infected by fungus disease also known as “Aspergillosis” [1–9]. Bio active compounds with antifungal activities disable fungus strains by targeting key components of fungal metabolism like cell-walls enzymes. Studies revealed that drugs targeting fungal cell wall components like chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase can be promising antifungal therapeutic agents as no such structure exits in humans [15–18]. Since cell wall-based enzymes are pivotal for fungus survival, it is essential to exploits them as a key target of anti-fungal agent. Hence, chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase may offer a new active fungicidal approach to treat “Aspergillosis”. In view of this, the molecular docking study was carried out to examine the binding interactions of 1,8 cineole with chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase.

3.1 Molecular Docking

Among all, structure-based drug design (SBDD) is most commonly used, which is based on 3-D structure [22]. In SBDD, Molecular docking is a key technique that can be applied in designing drug-making process. In-silico docking has facilitated researchers to monitor conformations and affinities of a collection of bio-actives against receptors [23]. This study investigated the docking of 1,8 cineole bioactive molecule from eucalyptus oil as key fungal inhibitor candidate against chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase enzymes. In-silico docking results based on docking score and area are demonstrated in Table 1. Among all enzymes, it was found that Glucosamine-6-phosphate synthase depicted strong docking with 1,8 cineole as evident from dock score of 3670. Docking score for chitin synthase and UDP-glycosyltransferase was 3278 and 3232, respectively. Docking pose and molecular interactions of 1,8 cineole with chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase are shown in Fig. 1. It was observed that 1,8 cineole successfully docked in the active sites of with chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase. Chitin synthase is a membrane bound enzyme complex having three domains: an N-terminal domain, a catalytic domain and a C-terminal trans-membrane domain. From in-silico analysis it was found that 1,8 cineole exhibited its interaction with catalytic domain involved in chitin chain elongation between
Table 1 Molecular docking of fungal receptors

| Fungal Receptor                  | Dock score | Dock score Area | Dock score ACE | Dock score Transformation | Interacting residues within 4 Å radius | Hydrogen bonds |
|---------------------------------|------------|-----------------|----------------|---------------------------|---------------------------------------|----------------|
| Chitin synthase                 | 3278       | 344.70          | − 55.88        | 0.87 1.42 2.56 − 4.58 − 29.63 13.46 | GLN412                                | PRO48, 131, TRP306 |
| UDP-glucosyltransferase         | 3232       | 351.70          | − 66.24        | 0.24 0.58 2.74 74.99 81.74 28.34 | −                                      | LYS334, VAL356 |
| Glucosamine-6-phosphate synthase| 3670       | 387.30          | − 106.05       | − 1.58 1.13 0.68 3.05 32.01 68.97 | THR302                                | HIS504, SER401 |

Fig. 1 Molecular docking of 1,8 cineole with fungal cell wall receptors
N and C terminal domains [24]. Glucosamine-6-phosphate synthase posse N-terminal and C-terminal ones, catalyzing glutamine hydrolysis and sugar-phosphate isomerization [25, 26]. Computational analysis revealed that 1,8 cineole successfully docked with C terminal domain involved in sugar-phosphate isomerization. UDP-glycosyltransferase enzyme has two-domain structures: N-domain for the binding site of the aglycone substrate and C-terminal likely site of UDP-glucose binding [27]. The docking analysis exhibited interaction of 1,8 cineole with C-terminal UDP-glucose binding domain. These results are in agreement with those of [28, 29] as they stated that molecular docking analyses were performed to clarify the antifungal effectiveness of the most active compounds of essential oil from *Trachyspermum ammi*, *Thymus vulgaris* and *Boswellia carteri* against fungal enzymes. This study indicates that eucalyptus essential oil may be considered as the most important sources of antifungal compounds.

During docking drug molecule either forms hydrophobic interactions or hydrogen bonding with in the active site residues of receptor that determines affinity of ligand with receptor. So molecular interactions of 1,8 cineole with chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase were further evaluated. It was observed that the interaction of 1,8 cineole in active sites of chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase was mediated by both hydrophobic and hydrogen bond interactions. With Glucosamine-6-phosphate synthase, hydrophobic interactions were observed via HIS 504 (Fig. 2). Hydrogen bond interactions of 1,8 cineole with Glucosamine-6-phosphate synthase were also observed via SER 401. For chitin synthase, hydrophobic interactions were observed via PRO48, 131, TRP306. With UDP-glucosyltransferase, hydrophobic interactions were observed via LYS334 and VAL336. No hydrogen bond interactions were observed with chitin synthase, UDP-glycosyltransferase. Greater the hydrogen bonds between the enzyme and ligand determines the strength of binding [30]. In view of this, due to having hydrogen bond interaction, 1,8 cineole depicted strong binding with Glucosamine-6-phosphate synthase as compared to other enzymes which was also evident from its docking score. Active site prediction by CAST-P server indicated interacting residues in the major cavity of chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase enzymes (Table 2). With CASTp, a major pocket was identified with Area (SA) of 939 and Volume (SA) of 3369 in chitin synthase. While Area (SA) of 1648 and Volume (SA) of 1198 were observed for Glucosamine-6-phosphate synthase. Since 1,8 cineole poses high affinity towards chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate enzymes so it was postulated that chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate proteins becomes closed upon binding with 1,8 cineole that in turn induces a conformational change in chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate proteins and stop further execution of catalysis of fungal cell wall synthesis hence down-regulate the infectivity of fungus into host cell. The present study results were in consonance with the earlier *in-silico* findings suggesting that polypharmacological agents via cell wall inhibition can act as a therapeutic for the management of Aspergillosis among COVID-19 patients [31, 32].

### 3.2 PASS analysis, *In-silico* Bioactivity, Cell Toxicity and ADMET Properties

For therapeutic use of drugs in living organisms, ADMET properties (absorption, distribution, metabolism, excretion
and toxicity) are very imperative for the success of any drug [29]. To find out drug likeliness, the Lipinski rule of 5 (RO5) is generally used. It is based on some molecular parameters like TPSA (polar surface area), mlog P (partition coefficient), number of hydrogen bond donors, molecular weight and number of hydrogen bond acceptors. According to this rule for drug-like properties ligands should have log P ≤ 5, number of H-bond acceptors ≤ 10, and H-bond donors ≤ 5 and no more than 1 violation. As shown in Table 3, 1,8 cineole has shown good agreement with the given criteria. Hence, it was postulated that bioactive compound 1,8 cineole could be considered an oral drug [29]. In-silico absorption of 1,8 cineole was 100%. It was observed that 1,8 cineole was a low molecular weight ligand. It was cited that low MWT compounds are easily diffused and transported across the biological membranes as compared to high MWT compounds [30]. The Log $P_{o/w}$ value was also in acceptable range. In rational drug design and pharmacokinetic analysis, Log $P_{o/w}$ is a key parameter to assess the lipophilicity of any drug and its distribution in the body after absorption [29, 30]. The surface view depicting molecular lipophilicity potential (MLP) is also shown in Fig. 3. MLP is convenient property to rationalize numerous molecular ADME characteristics (for example: plasma-protein binding or membrane penetration). Analysis of 3D distribution of hydrophobicity on molecular surface is predominantly helpful when explaining differences are observed in ADME properties of molecules with the same Log $P_{o/w}$ [28–31]. The topological polar surface area (TPSA) value was 9.23 Å squared. Topological polar surface area is a key predictor of drug transport properties such as nice permeability, bioavailability and intestinal absorption [28–31]. GI (Gastrointestinal tract absorption) of 1,8 cineole was high (Table 3). In order to exert a toxic effect, drug molecules have to be absorbed from intestinal tract in the body. Further, 1,8 cineole was non substrate to efflux transporters such as P-glycoprotein (P-gp). In the gut, P-glycoprotein pumps drugs back into the lumen, decreasing their absorption [33]. 1,8 cineole bioactive compound elaborated non-inhibitory potential against CYP450 series of enzymes, involved in liver detoxification in body [34, 35]. These observations indicated that 1,8 cineole can easily interact with target receptors and can be further taken in the evaluation of biological activity score.

| Pdb id | Macromolecule | Native ligand | Interacting Active site residues | Cavity |
|--------|---------------|--------------|---------------------------------|--------|
| 4gf8   | ETH362        | TYR 454, ASP458, THR456,457, PRO48, 131, LYS459, ASN129, PHE40, SER128, TYR 132,454, TRP396, ALA457, GLN412 | | 939.053        | 3369.091 |
| 5u6m   | ETH362        | ASN333, 332, LEU 331, PHE354, LYS415, 419, 334, ARG422, SER332, ALA335, VAL 356 | | 1087.766       | 1096.959 |
| 1jxa   | ETH362        | ALA400, VAL399, GLY 505, HIS 504, TYR 304, GLN348, SER 303, LEU 346, LYS 603, GLU 396, GLN 348, CYS300, THR352, 302, GLU 396 | | 1648.961       | 1198.342 |
### Table 3  Physicochemical Properties and Pharmacokinetics properties of 1,8 cineole

| Property                                      | Value            |
|-----------------------------------------------|------------------|
| **Physicochemical properties**                |                  |
| Formula                                       | C10H18O          |
| Molecular weight                              | 154.25 g/mol     |
| Num. heavy atoms                              | 11               |
| Num. arom. heavy atoms                        | 0                |
| Fraction Csp³                                  | 1.00             |
| Num. rotatable bonds                          | 0                |
| Num. H-bond acceptors                         | 1                |
| Num. H-bond donors                            | 0                |
| Molar Refractivity                            | 47.12            |
| TPSA                                          | 9.23 Å²          |
| **Lipophilicity**                             |                  |
| Log $P_{ow}$ (iLOGP)                          | 2.58             |
| Log $P_{ow}$ (XLOGP3)                         | 2.74             |
| Log $P_{ow}$ (WLOGP)                          | 2.74             |
| Log $P_{ow}$ (MLOGP)                          | 2.45             |
| Log $P_{ow}$ (SILICOS-IT)                     | 2.86             |
| Consensus Log $P_{ow}$                        | 2.67             |
| **Pharmacokinetics**                          |                  |
| GI absorption                                 | High             |
| BBB permeant                                  | Yes              |
| P-gp substrate                                | No               |
| CYP1A2 inhibitor                              | No               |
| CYP2C19 inhibitor                             | No               |
| CYP2C9 inhibitor                              | No               |
| CYP2D6 inhibitor                              | No               |
| CYP3A4 inhibitor                              | No               |
| Log $K_p$ (skin permeation)                   | ~ 5.30 cm/s      |
| **Druglikeness**                              |                  |
| Lipinski                                      | Yes; 0 violation |
| Ghose                                         | No; 1 violation: MW < 160 |
| Veber                                         | Yes              |
| Egan                                          | Yes              |
| Muegge                                        | No; 2 violations: MW<200, Heteroatoms<2 |
| Bioavailability Score                         | 0.55             |
| **Medicinal chemistry**                       |                  |
| PAINS                                         | 0 alert          |
| Brenk                                         | 0 alert          |
| Leadlikeness                                  | No; 1 violation: MW<250 |
| Synthetic accessibility                       | 3.65             |
Biological activity is a key parameter which describes the effect of a drug in living systems. In living systems, ligands have to be bound to biological targets which are also known as drug targets [36]. Drug targets mostly include common proteins such as enzymes, receptors and ion channels. Bioactivity score was calculated with online Molinspiration software based on following parameters such as binding to GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor. This score as per rule is calculated in three different ranges: score > 0, drug is active, if it is between −5.0 and 0, drug is judiciously active and if score < than −5.0, drug is quiet. For 1,8 cineole, bioactivity score for Ion channel modulator was 0.01 whereas for GPCR ligand, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor score was in the range of −5.0 and 0, (Table 4). All these observations indicated that 1,8 cineole possess such properties as are required for the bio active molecules to act as potential drugs. Similar observations have been reported by researchers working on different drug formulations [36, 37]. The bioactivity score deliver the evidence about the binding cascade of the 1,8 cineole that is used for the improvement of a new functional drug with increased binding selectivity profile and less undesirable effects [35, 36]

For pharmaceutical industries, proper risk assent of a chemical drug is a prerequisite to assess the safety profile of a therapeutic drug [38]. In this regard, in silico toxicity is a key platform to evaluate toxicity prediction of drugs that could be detrimental to humans, animals, and environments [39]. Thus toxicity profile of 1,8 cineole was evaluated and toxicity profile revealed that 1,8 cineole bioactive molecule was mostly non toxic to organs as inactive prediction was observed like hepatotoxicity (Table 5). Drug-induced hepatotoxicity is the major reason for liver damage and the main reason for the un-success of major drugs in the market [40]. Further, 1,8 cineole was non-carcinogenic and non-mutagenic. Mutagenic nature of biomolecules is harmful to cell and is the main reason behind certain diseases, e.g. cancer [41]. Further 1,8 cineole showed inactiveness towards targets-based on biological pathways like Nuclear receptor signaling pathways and Stress response pathways. All these targets like aryl hydrogen receptor (AhR), androgen receptor (AR), androgen receptor ligand binding domain (AR-LBD), 2/antioxidant responsive element (ARE), heat shock factor response element (HSE), mitochondrial membrane potential (MMP) are important components of biological system inside human body [42]. Toxicity radar chart is also shown in Fig. 4, that quickly exemplifies the assurance of positive toxicity outcomes compared to the average of its class. Further, 1,8 cineole depicted cell line toxicity to tumor cell lines (Table 6). As per Way2Drug server prediction, it was found that values of Pa > values of Pi, depicted that 1,8 cineole 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.

3.3 In vitro Antifungal Activity of Eucalyptus Oil

In order to validate the in-silico findings, a wet-lab experiment was designed to evaluate the antifungal potential of eucalyptus oil against two fungal strains: Aspergillus niger and Aspergil- lus oryzae. In both fungal strains, complete mycelial growth inhibition was observed at essential oil concentration of 30 μL/mL (Fig. 5, Table 7). Eucalyptus essential oil depicted significant antifungal activity (mycelial growth inhibition was 40–50%) against Aspergillus oryzae and Aspergillus niger at a concentration of 5 μl/ml after 5 days of incubation.
Full mycelial growth inhibition (100%) was observed at a concentration of 30 μl/ml. A substantial antifungal activity was also observed with positive control streptomycin which was reported to be a fungistatic or fungicidal [43]. The strong antifungal activity of eucalyptus essential oil may be due to the richness of eucalyptol. These observations were in consonance with earlier studies showing the antifungal effect of methanolic extracts of eucalyptus essential oil against Alternaria alternate, a pathogenic fungus causing leaf spot infection in plants [44]. Antifungal effect of eucalyptus essential oil has been reported against other pathogenic fungi strains like: Penicillium digitatum, Fusarium solani, Colletotrichum gloeosporioides, Pythium ultimum, Rhizoctonia solani, Bipolaris sorokiniana, Fusarium graminearum, and Fusarium sporotrichioides [45, 46].

| Classification                | Target                                      | Prediction | Probability |
|-------------------------------|---------------------------------------------|------------|-------------|
| Organ toxicity                | Hepatotoxicity                              | Inactive   | 0.86        |
| Toxicity end points           | Carcinogenicity                             | Inactive   | 0.68        |
| Toxicity end points           | Immunotoxicity                              | Inactive   | 0.99        |
| Toxicity end points           | Mutagenicity                                | Inactive   | 0.96        |
| Toxicity end points           | Cytotoxicity                                | Inactive   | 0.75        |
| Tox21-Nuclear receptor signalling pathways | Aryl hydrocarbon Receptor (AhR) | Inactive   | 0.98        |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor (AR)                      | Inactive   | 0.99        |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor Ligand Binding Domain (AR-LBD) | Inactive | 1.0         |
| Tox21-Nuclear receptor signalling pathways | Aromatase                                   | Inactive   | 0.98        |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Alpha (ER)                | Inactive   | 0.96        |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Ligand Binding Domain (ER-LBD) | Inactive | 0.97        |
| Tox21-Nuclear receptor signalling pathways | Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma) | Inactive | 0.99        |
| Tox21-Stress response pathways | Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) | Inactive | 0.99        |
| Tox21-Stress response pathways | Heat shock factor response element (HSE)    | Inactive   | 0.99        |
| Tox21-Stress response pathways | Mitochondrial Membrane Potential (MMP)      | Inactive   | 0.89        |
| Tox21-Stress response pathways | Phosphoprotein (Tumor Suppressor) p53       | Inactive   | 0.99        |
| Tox21-Stress response pathways | ATPase family AAA domain-containing protein 5 (ATAD5) | Inactive | 0.99        |

Probability (P) indicates toxicity confidence score of the 1,8 cineole. score > 1: toxic (active)
**Fig. 4** Toxicity radar chart of 1,8 cineole

**Table 6** 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")
4 Conclusion

Aspergillosis has emerged as a pandemic in India. This study findings emanated from both in silico and in vitro revealed that eucalyptus essential oil due to the richness of eucalyptol or 1,8 cineole from eucalyptus essential oil plant could be promising antifungal therapeutic agents against chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase protein.

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Declarations

Conflict of interest The authors declares that they have no competing interest.

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