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Molecular docking of a bioactive compound of *C. sinensis* n-heptadecanol-1 with opportunistic fungi

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**ABSTRACT**

With the expansion of immune-impaired people during second wave of Covid-19 the case of mucormycosis and aspergillosis caused by different fungi such as *Rhizopus oryzae*, *Candida albicans* and *Aspergillus niger* arose in different states. The current study reveals the molecular interaction of ligand and protein to find the novel and natural drug. The molecular docking was carried out by CB-dock tool and the ligand compound n-heptadecanol-1 were docked with different protein of opportunistic fungi. The most significant outcomes were depicted against *C. albicans* (~4.6 kcal/mol) followed by *R. oryzae* (~3.9 kcal/mol) and *A. niger* (~3.0 kcal/mol). n-heptadecanol-1 showed therapeutic potential and eliminates the issue of drug inadequacy that could act as potential ant-fungal agent.

1. Introduction

Many fungal infections are known to infect the human body both externally and internally due to several reasons. One of the causes is the weak inherent immune system and immunocompromised people due to diabetes, AIDS and drug users, cancer patients and COVID-19 patients. In 2021, second wave of coronavirus surprisingly the COVID-19 patients were infected by fungal pathogens causing mucormycosis, opportunistic candidiasis and aspergillosis.

At present, these opportunistic fungi have become more offensive to the COVID-19 patients and immunocompromised individuals. Several recent cases of mucormycosis have been observed worldwide with a mortality rate of approximately 80% \cite{1}. Mucormycosis is caused by the members of Mucorales e.g. the species of *Mucor*, *Rhizopus*, *Rhizomucor* and *Absidia* etc. Mucormycosis is characterized by nodular lesions and inflammation leading to tissues undergoing extensive bruise, formation of ulcers and finally proclamation. Existential and experimental evidences undeniable point to phagocytes play a significant role as the primary host defence against mucormycosis. People with an ultra-low of phagocyte number or impaired phagocyte function have a substantial risk for invasive mucormycosis infection. Normal and healthy immune cells, such as mononuclear cells and polymorphonuclear phagocytes readily take up and kill the hyphae and spores of the fungi that cause mucormycosis. Neutropenia induced by cytotoxic chemotherapy is also a well-known risk factor for mucormycosis \cite{2}. Mucormycosis has a distinct proclivity for invasion of endothelial cells of the vascular system, and this ability is likely important in the propagation of disease from a primary site of infection \cite{3}.

Mucormycosis cause infection such as gastrointestinal tract, pulmonary infection, cutaneous, rhino-orbital cerebral infection cause in diabetic and organ transplant persons and immunosuppressive people \cite{3}.

Among patients with COVID-19 who require hospitalisation, many suffer from severe respiratory distress syndrome, are admitted to an ICU and are exposed to various factors associated with candidemia \cite{4}. Therefore, candidemia could be a potential complication of patients with COVID-19 cared in ICUs. In Italy reported 21 cases of candidemia in patients with COVID-19 and found a higher incidence of candidemia in COVID-19 patients compared with a historical cohort \cite{5}. Similarly, in India patients are also endure from fatal disease Candiasis.

Aspergillus genus is known for causing invasive pulmonary aspergillosis (IPA) as a common complication in patients with severe respiratory affliction and high mortality rates is also increased \cite{6}. There are many incline factors to the development of IPA, basically recognized in protracted treatment with corticosteroids and lung epithelial damage \cite{7}. Several cases of IPA have been documented as super-infections in patients with severe respiratory illness such as influenza and MERS-CoV \cite{8}. Starting in December 2019 many severe respiratory syndrome cases caused by Coronavirus-19 (SARS-CoV-2) have been diagnosed. The
clinical impact of this infection defines a highly dysregulated immune response and diffuse lung damage, which lead to the early onset of secondary infections. Here we describe a case of invasive pulmonary aspergillosis caused by Aspergillus niger in a patient with COVID-19 pneumonias and acute respiratory distress syndrome.

The antifungals comprise Amphotericin B, Echinocandins and Posaconazole that are nephrotoxic and hepatotoxic [9]. The drugs are mostly used synergistically, but owing to the emergence of drug-resistant fungal strains, their varied sensitivity towards antifungal agents [10]. Due to the more use of anti-fungal drugs and therapies, the microorganisms show resistance regarding these drugs. Therefore, medicinal plants play a significant role to overcome such complications. Camellia sinensis is the medicinal plant having antimicrobial and antioxidant property due to the presence of active compounds such as flavonoids, terpenoids, tannins, saponins and alkaloids [11]. So, the utilize of C. sinensis to impede the fungal growth. This research article focuses on the antifungal activity of bioactive compounds extracted from Camellia sinensis against opportunistic fungi.

2. Material and methods

The bioactive compound (ligand) n-heptadecanol-1 was procured from our previous results of GC-MS analysis [12].

The crystal structure of compounds was obtained from the PubChem database (n-heptadecanol-1). The protein structure of ALS-3 of Candida albicans, G-6-P of Rhizopus oryzae and Est A of Aspergillus niger was obtained from Protein Data Bank (PDB, https://www.rcsb.org/), the high-resolution model was selected. Then, the docking was performed by the CB-Dock tool [13].

3. Results

3.1. Molecular docking of n-heptadecanol-1 with opportunistic fungi

The n-heptadecanol-1 was docked with proteins of various opportunistic and pathogenic fungi. n-heptadecanol-1 was docked with protein of R. oryzae by CB-dock blind tool, which aids in predicting the exquisite binding position of ligand and protein. The generating expounding of protein-ligand binding is explicable for its activity. The binding affinity was found −3.9 kcal/mol. Similarly, the same compound binds with the protein of C. albicans, and A. niger the binding affinity was found −4.6 kcal/mol and −3.0 kcal/mol (Tables 1-3). The best binding affinity of protein was exhibited with C. albicans followed by R. oryzae and A. niger.

| S. No. | Ligand Binding Affinity | RMSD/upper bound | RMSD/lower bound |
|-------|------------------------|------------------|------------------|
| 1.    | Fob1 & Fob2 + HEP      | −3.9             | 0.000            |
| 2.    | Fob1 & Fob2 + HEP      | −3.8             | 1.670            | 4.838            |
| 3.    | Fob1 & Fob2 + HEP      | −3.7             | 1.593            | 3.908            |
| 4.    | Fob1 & Fob2 + HEP      | −3.5             | 1.778            | 2.777            |
| 5.    | Fob1 & Fob2 + HEP      | −3.5             | 2.793            | 4.855            |

Molecular docking of ligand with protein: A. HEP+ Fob1; B. HEP+ ALS-3; C. HEP+ EstA.
4. Discussion

*Camellia sinensis* is a known medicinal plant that contains various bioactive compounds. Among these bioactive compounds, n-heptadecanol-1 was found more potent in our previous study. This compound is known for its antimicrobial and anti-inflammatory activity [12,14]. Therefore, n-heptadecanol-1 was used as a ligand to identify the potency of any compound that is used as a drug for prospects. Similarly, n-heptadecanol is docked with the protein of opportunistic fungi that infects immunocompromised people and nowadays especially covid patients.

*R. oryzae* is the main causative agent of mucormycosis. The study of germination conidia and adherence to plastic revealed that attachment occurred before germination and decreased dramatically with germ tube formation. This relates to the important role of the cell wall of the fungus concerning both carbohydrate composition and distribution of anionic sites. Furthermore, the attachment of spores to extracellular matrix components immobilized onto wells of polystyrene microtiter plates has been investigated. Spores adhered readily to immobilized laminin or type IV collagen, but not to fibronectin or the glycosaminoglycans. The immunofluorescence technique revealed that laminin and type IV collagen interacted exclusively with spores and mother cells of germ tubes. Thus, the identification of laminin or collagen by spores may participate in their adherence to epithelial basement membranes exposed after epithelial tissue damage which frequently accompanies the pre-disposing factors for mucormycosis [15]. It was reported that *C. sinensis* have polyphenols that depicted anti-fungal potential against the species of *Rhizopus* and *Candida* [16]. In our study, the protein of *R. oryzae* docked with ligand (n-heptadecanol-1) depicted good docking outcomes and can inhibit the growth of *R. oryzae*. So, it is an effective and natural way to impede the growth of *R. oryzae*. The binding affinity was found as −3.9 kcal/mol.

*C. albicans* play a role in normal human flora, and it grows on mucosal surfaces in well-being individuals. In susceptible and immune impaired hosts, these fungi can cause both hematogenous and mucosal and disseminated ailment. It persists in the host tissue and induces disease, it must be capable to attach both biotic and abiotic surfaces, invade host cells, and acquire iron for growth. The *C. albicans* hypha-specific surface protein Als3 is a member of the agglutinin-like sequence (Als) family of proteins and is important for adhesion and invasion. Functioning as an adhesin, Als3 mediates attachment to epithelial cells, endothelial cells, and extracellular matrix proteins. It also plays a significant role in biofilm formation on prosthetic surfaces, both alone and in mixed infection with *S. gordonii*. Als3 is one of two known *C. albicans* invasions. It binds to host cell receptors such as E-cadherin and N-cadherin and thereby induces host cells to endocytose the organism. Als3 also binds to host cell ferritin and enables *C. albicans* to utilize this protein as a source of iron. Because of its multiple functions and its high expression level, *C. albicans* is recognized as a promising target for vaccines that induce protective cell-mediated and antibody responses. In our study, we target this protein (Als3) to inhibit the activity of *C. albicans* that are harmful to immunocompromised people. The protein of *C. albicans* docked with ligand showed remarkable outcomes. It showed good binding affinity −4.6 kcal/mol energy. Anti-fungal activity was also reported against *C. albicans* [17]. In our previous study, n-heptadecanol-1 showed a good zone of inhibition against *C. albicans* [12].

*A. niger* is a non-pathogenic opportunistic fungus, even though patients with a record of severe ailment and immunocompromised treatment can favour the growth of fungi. It is an opportunistic human pathogen and important for its epidemiological studies. It causes deadly fungal air-borne infection in advanced countries known as invasive pulmonary aspergillosis, a fat disease caused by mycosis-infected immunocompromised people [18]. At present time, these fatal fungi also infect the immunocompromised covid patients and show their severe and deadly symptoms. EstA is a protein and remarkable to its specificity towards short acyl chain substrate and recognized as a new member of fungal esterases within the α/β hydrolase super protein of family that causes pathogenicity. The outcomes of our study depicted good binding affinity as −3.0 kcal/mol.

Therefore, it has been proved that the compound procured from *C. sinensis* is effective and able to impede the growth of such opportunistic fungi and replaces the synthetic antifungal drug. As per our knowledge, this is the first report and such study has not been done by anyone so far.

5. Conclusion

Nowadays the opportunistic fungi are becoming fatal for immunocompromised patients which the COVID-19 cases increasing with several wave. The increasing incidence and severity of invasive fungal mycoses have led to new and natural anti-fungal strategies aimed to reinstate synthetic drugs. Our study showed an alternatives and substitute ways to shut down the growth and development of opportunistic fungi effectively.

**Ethical statement**

Not Applicable.

**Consent to participate**

Not Applicable.

**Consent to publish**

Yes.

**Author contribution**

Investigation, Methodology and Writing the original draft of the manuscript: Surbhi Pradhan; Methodology and Supervision: Prof. R.C. Dubey. All authors read and approved the final manuscript.

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All data have been provided.

CRediT authorship contribution statement

Surbhi Pradhan: Investigation, Methodology, and, Writing – original draft, of the manuscript. All authors read and approved the final manuscript. R.C. Dubey: Methodology, and, Supervision, All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no conflict of interest.

References

[1] J.A. Ribes, C.L. Vanover-Sams, D.J. Baker, Zygomycetes in human disease, Clin. Microbiol. Rev. 13 (2) (2000) 236–301, pmid:10756000.
[2] A.R. Waldorf, N. Ruderman, R.D. Diamond, Specific susceptibility to mucormycosis in murine diabetes and bronchoalveolar macrophage defense against Rhizopus, J. Clin. Invest. 74 (1984) 150–160.
[3] T.T. Riley, C.A. Muzny, E. Swiatlo, D.P. Legendre, Breaking the mold: a review of mucormycosis and current pharmacological treatment options, Ann. Pharmacother. 50 (9) (2016) 747–757.
[4] P. Eggimann, J. Garbino, D. Pittet, Epidemiology of Candida species infections in critically ill non-immunosuppressed patients, Lancet Infect. Dis. 3 (2003) 685–702.
[5] M. Nucci, G. Barreiros, L.F. Guimarães, V.A. Deriquehem, A.C. Castineiras, S.A. Neuter, Increased incidence of candidemia in a tertiary care hospital with the COVID-19 pandemic, Mycoses 64 (2) (2021) 152–156.
[6] L. Trovato, M. Calvo, G. Migliorisi, M. Astuto, F. Oliveri, S. Oliveri, Fatal VAP-related pulmonary aspergillosis by Aspergillus niger in a positive COVID-19 patient, Respiratory Medicine Case Reports 32 (2021) 101367.
[7] A. Mohamed, T.R. Rogers, A.F. Talento, COVID-19 associated invasive pulmonary aspergillosis: diagnostic and therapeutic challenges, J Fungi (Basel) 6 (3) (2020) 115.
[8] P. Koehler, O.A. Cornely, B.W. Bottiger, et al., COVID-19 associated pulmonary aspergillosis, Mycoses 63 (6) (2020) 528–534.
[9] M. Chayakkulkeeree, M.A. Ghanem, J.R. Perfect, Zygomyces: the re-emerging fungal infection, Eur. J. Clin. Microbiol. Infect. Dis. 25 (4) (2006) 215–229.
[10] U. Gupta, K. Banerjee, R. Gabrani, S. Goga, S.K. Sharma, C.K. Jain, Variability analyses of functional domains within glucosamine-6-phosphate synthase of mycoses causing fungi, Bioinformation 6 (5) (2011) 196.
[11] S. Pradhan, R.C. Dubey, Proximate analysis. Total phenolic content, antioxidant activity and anti-diabetic property of Camellia sinensis and Camellia assamica, Biochem. Cell. Arch. 20 (1) (2020) 15–23.
[12] S. Pradhan, R.C. Dubey, GC-MS analysis and molecular docking of bioactive compounds of Camellia sinensis and Camellia assamica, Arch. Microbiol. 203 (5) (2021) 2501–2510.
[13] Yang Liu, Maximilian Grimm, et al., CB-Dock: a web server for cavity detection-guided protein–ligand blind docking, Acta Pharmacol. Sin. (2019).
[14] M.J. Chavan, P.S. Wakte, D.B. Shinde, Analgesic and anti-inflammatory activity of caryophyllene oxide from Ammona squamoua L. bark, Phytomedicine 17 (2) (2010) 149–151.
[15] J.F. Bouchara, N.A. Oumeziane, J.C. Lissizky, et al., Attachment of spores of the human pathogenic fungus Rhizopus oryzae to extracellular matrix components, Eur. J. Cell Biol. 70 (1) (1996 May) 76–83.
[16] X. Yang, X. Jiang, Antifungal activity and mechanism of tea polyphenols against Rhizopus stolonifer, Biotechnol. Lett. 37 (7) (2015) 1463–1472.
[17] W.H. Yoon, J.H. Choi, K.H. Lee, C.H. Kim, Antimicrobial and antitumor activities of seed extracts of Camellia sinensis L, Korean Journal of Food Science and Technology 37 (1) (2005) 108–112.
[18] Y. Bourne, A.A. Haqer, H. Chahinian, M. Juin, I.H. De Graaff, P. Marchot, Aspergillus niger protein EstA defines a new class of fungal esterases within the α/β hydrolase fold superfamily of proteins, Structure 12 (4) (2004) 677–687.

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