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In-silico study of some natural compounds used as antifungal agents against *Candida albicans*

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ABSTRACT: Fungal diseases are very common these days, so there is a high need to design and develop new antifungal drugs that can counter these diseases. *Candida albicans* is one of the opportunistic pathogenic yeasts that can cause serious diseases such as oropharyngeal candidiasis, vulvovaginal (genital) candidiasis, and invasive candidiasis (candidemia). This article focuses on the in-silico evaluation of anti-candidal activity of some natural compounds like ajoene, allicin, curcumin, gingerol, nimbin, nimbolide, nimonol and 6-Shogaol. Binding affinity of these compounds have been determined against the most common targets in *C. albicans* viz. cytochrome p450, lanosterol synthase, serine/threonine protein kinase, squalene monooxygenase, sterol-14-demethylase and thymidylate synthase. PatchDock and FireDock web servers were used to carry out the docking studies. The proposed targets of ajoene, allicin, curcumin, gingerol, nimbin, nimbolide, nimonol and 6-Shogaol are sterol 14-demethylase, cytochrome p450, cytochrome p450, cytochrome p450, cytochrome p450, squalene monooxygenase, lanosterol synthase and squalene monooxygenase respectively based upon the binding energies obtained by the docking studies. This study opens new avenues in the usage of the natural compounds as potential antifungal agents.

Keywords: In-silico; *Candida albicans*; Candidiasis; Docking; Software.

1. INTRODUCTION

Fungi are the group of eukaryotic microorganisms that digest food externally and absorb nutrients directly through their cell wall. Most of the fungi reproduce by spores and have a body (thallus) comprising of microscopic tubular cells called as hyphae. Fungi are heterotrophs and saprophytic in nature [1]. Some common examples of fungi are *Candida* species, *Saccharomyces* species, *Aspergillus* species, *Cryptococcus* species etc.

Candida albicans exists in the yeast form of fungi. Multiple species of *Candida* are harmless commensals or endosymbionts of host including humans [2]. *C. albicans* majorly causes three types of infections including oropharyngeal candidiasis, vulvovaginal (genital) candidiasis, and invasive candidiasis (candidemia) [3].

In comparison to the available antibacterial drugs, the antifungal drugs are very less. This is primarily due to the shortage of the antifungal targets owing to the eukaryotic nature of the fungi [4]. The available antifungal drugs are not free from side effects like nephrotoxicity under prolonged usage, epigastric pain,

headache, loss of appetite etc. [5]. In order to overcome the scarcity of the broad spectrum antifungal drugs it is mandatory to search for potential drug candidates amongst the natural compounds. With this concept in mind, the current manuscript focuses on determining the probable antifungals targets of certain natural compounds having unknown targets using *in-silico* approach. This is also accompanied by studying their interaction with their respective antifungal targets. Natural compounds obtained from neem (*Azadirachta indica*), ginger (*Zingiber officinale*), garlic (*Allium sativum*) and turmeric (*Curcuma longa*) have been studied in this manuscript with reference to their antifungal properties against *C. albicans*.

Neem (*Azadirachta indica*) is a natural herb and its constituents have been demonstrated to exhibit anti-inflammatory, antiulcer, antimalarial, antifungal and anticarcinogenic properties. Amongst the different compounds present in neem, nimbin, nimbolide and nimonol are known to possess antifungal properties [6].

Garlic (*Allium sativum*) is known for its antibacterial nature, property of reducing the blood pressure, improving cholesterol levels and also as an antifungal agent. Garlic contains allicin and ajoene compounds which have the ability to fight against the fungal pathogens [7, 8].

Turmeric and ginger (*Zingiber officinale*) both belong to the Zingiberaceae family and are rhizomes. They both have anti-inflammatory and antifungal properties. Turmeric is also known for its healing properties. The main active compound present in turmeric is curcumin [9].

Ginger is loaded with nutrients and bioactive compounds such as gingerol and 6-Shogaol which are very beneficial and also work against the fungal pathogens like *C. albicans* [10].

These natural antifungal compounds have been tested for their interaction and activity against some commonly known targets present in *C. albicans* viz. cytochrome p450, lanosterol synthase, serine/threonine protein kinase, squalene monooxygenase, sterol 14 demethylase and thymidylate synthase in order to ascertain the anti-candidal activity of these compounds and their most probable target.

2. MATERIALS AND METHODS

2.1. Sequence retrieval

The National Centre for Biotechnology Information (NCBI) was used to retrieve sequence data of cytochrome p450 (CAA21953.1), lanosterol synthase (AOW27286.1), squalene monooxygenase (AOW26497.1), thymidylate synthase (AOW27536.1), sterol 14-demthylase (AOW29509.1) and serine/threonine protein kinase (AOW28668.1) proteins.

2.2. Homology modelling

Phyre^2 is a web based service which was used for the purpose of protein structure prediction [11]. FASTA sequences obtained from NCBI were utilized to predict the structure of cytochrome p450, lanosterol synthase, serine/threonine protein kinase, squalene monooxygenase, sterol 14 demethylase, and thymidylate synthase.

2.3. Ligand structure retrieval

PubChem (https://pubchem.ncbi.nlm.nih.gov) is a database which provides information about chemical molecules and their activities against biological assays. It is maintained by NCBI. In the current study it was used to retrieve the 3D structures of ajoene, allicin, curcumin, gingerol, nimbin, nimbolide, nimonol and 6-Shogaol in SDF format.

2.4. OpenBabel

OpenBabel [12] is downloadable software which is mainly used to interconvert chemical file formats. In this study, the SDF format of 3D structure of compounds (ajoene, allicin, curcumin, gingerol, nimbin, nimbolide, nimonol and 6-Shogaol) was converted into the PDB format using this software.

2.5. PatchDock and FireDock

Patchdock and FireDock [13, 14] are online web servers which have been used in this study for the purpose of molecular docking. Using the docking studies, the best suited targets for all the ligands (ajoene, allicin, curcumin, gingerol, nimbin, nimbolide, nimonol and 6-Shogaol) have been proposed.

2.6. UCSF Chimera

UCSF Chimera [15] is an extensible program for interactive visualization and analysis of molecular structure and related data, including density maps, supramolecules assemblies, sequence alignment, docking results and conformational ensembles. In this study, the results obtained by the FireDock were visualized and analyzed using chimera.

2.7. RAMPAGE

Rampage [16] is the web server which is used to check the number of residues in favoured region, allowed region and outlier regions of the proteins. Cytochrome p450, lanosterol synthase, serine/ threonine protein kinase, squalene monooxygenase, sterol 14 demethylase and thymidylate synthase were validated for their modelled structure using RAMPAGE.

3. RESULTS

The sequences of the proteins which were obtained from NCBI are cytochrome p450, lanosterol synthase, serine/ threonine protein kinase, squalene monooxygenase, sterol 14 demethylase and thymidylate synthase. The proteins along with their respective sequences in FASTA format have been mentioned in Table 1.

Phyre[2] was used to determine the homology modelled structure of cytochrome p450, lanosterol synthase, serine/threonine protein kinase, squalene monooxygenase, sterol 14-demethylase and thymidylate synthase as shown in Figure 1. The relevant parameters (Confidence and Coverage) of all the models have been shown in Table 2.

Table 1. Proteins along with their respective amino acid sequences obtained from NCBI.

Proteins	FASTA Sequence
Cytochrome P450	>CAA21953.1 cytochrome P450 [*Candida albicans*] MNSTEVDNLPFQQQLTSFVELAVAKATGSPITTLFTIIFLILSYDQLSYQINKGSIAGPRFKFYPI IGPFLESLDPKFEEYKAKWDSGELSCVSIFHKFVVIASSRDLARKILSSPKYVKPCVVDVAIKI LRPTNWVFLDGKQHTDYRRSLNGLFSSKALEIYIPVQEKYMDIYLERFCKYDGPREFFPEFR ELLCALSLRTFCGDYITEDQIALVADNYYRVTAALELVNFPIIIPYTKTWYGKKIADDTMKIFE NCAAMAKKHINENNGTPKCVMDEWIHLMKEAREKHSEDPDSKLLVREFSNREISEAIFTFLF ASQDASSSLACWLFQIVADRPDIVAKIREEQLRVRNNNPDVRLSLDLINEMTYTNNVVKESL RYRPPVLMVPYVVKKSFPVTESYTAPKGAMIIPTLYPALHDPEVYDEPDSFIPERWENASGD MYKRNWLVFGTGPHVCLGKNYVLMLFTGMLGKFVMNSDMIHHKTDLSEEIKVFATIFPKD DLILEWKKRDPLKSL

Proteins	FASTA Sequence
Lanosterol Synthase	>AOW27286.1 lanosterol synthase [*Candida albicans* SC5314] MYYSEEIGLPKTDISRWRLRSDALGRETWHYLSQSECESEPQSTFVQWLLESPDFPSPPSSDI HTPDEAARKGADFLKLLQLDNGIFPCQYKGPMFMTIGYVTANYYSKTEIPEPYRVEMIRYIV NTAHPVDGGWGLHSVDKSTCFGTTMNYVCLRLLGMEKDHPVLVKARKTLHRLGGAIKNP HWGKAWLSILNLYEWEGVNPAPPELWRLPYWLPIHPAKWWVHTRAIYLPLGYTSANRVQ CELDPLLKEIRNEIYVPSQLPYESIKFGNQRNNVCGVDLYYPHTKILDFANSILSKWEAVRPK WLLNWVNKKVYDLIVKEYQNTEYLCIAPVSFAFNMVVTCHYEGSESENFKKLQNRMNDV LFHGPQGMTVMGTNGVQVWDAAFMVQYFFMTGLVDDPKYHDMIRKSYLFLVRSQFTENC VDGSFRDRRKGAWPFSTKEQGYTVSDCTAEAMKAIIMVRNHASFADIRDEIKDENLFDAVE VLLQIQNVGEWEYGSFSTYEGIKAPLLLEKLNPAEVFNNIMVEYPYVECTDSSVLGLTYFAK YYPDYKPELIQKTISSAIQYILDSQDNIDGSWYGCWGICYTYASMFALEALHTVGLDYESSS AVKKGCDFLISKQLPDGGWSESMKGCETHSYVNGENSLVVQSAWALIGLILGNYPDEEPIK RGIQFLMKRQLPTGEWKYEDIEGVFNHSCAIEYPSYRFLFPIKALGLYKNKYGDKVLV
Squalene monooxygenase	>AOW26497.1 squalene monooxygenase [*Candida albicans* SC5314] MSSVKYDAIIIGAGVIGPTIATAFARQGRKVLIVERDWSKPDRIVGELMQPAGIKALRELGMI KAINNIRAVDCTGYYIKYYDETITIPYPLKKDACITNPVKPVPDAVDGVNDKLDSDSTLNVD DWDFDERVRGAAFHHGDFLMNLRQICRDEPNVTAVEATVTKILRDPSDPNTVIGVQTKQPS GTVDYHAKLTISCDGIYSKFRKELSPTNVPTIGSYFIGLYLKNAELPAKGKGHVLLGGHAPA LIYSVSPTETRVLCVYVSSKPPSAANDAVYKYLRDNILPAIPKETVPAFKEALEERKFRIMPN QYLSAMKQGSENHKGFILLGDSLNMRHPLTGGGMTVGLNDSVLLAKLLHPKFVEDFDDHQ LIAKRLKTFHRKRKNLDAVINTLSISLYSLFAADKKPLRILRNGCFKYFQRGGECVNGPIGLL SGMLPFPMLLFNHFFSVAFYSVYLNFIERGLLGFPLALFEAFEVLFTAIVIFTPYLWNEIVR
Thymidylate Synthase	>AOW27536.1 thymidylate synthase [*Candida albicans* SC5314] MTVSPNTAEQAYLDLCKRIIDEGEHRPDRTGTGTKSLFAPPQLRFDLSNDTFPLLTTKKVFSK GIIHELLWFVAGSTDAKILSEKGVKIWEGNGSREFLDKLGLTHRREGDLGPVYGFQWRHFG AEYKDCDSDYTGQGFDQLQDVIKKLKTNPYDRRIIMSAWNPPDFAKMALPPCHVFCQFYV NFPTSSPDPNNPKQAKTAKPKLSCLLYQRSCDMGLGVPFNIASYALLTKMIAHVVDMDCGE FIHTLGDAHVYLDHIDALKEQFERIPKQFPKLVIK EERKNEIKSIDDFKFEDFEIVGYEPYPPIKMKMSV
Sterol 14-Demethylase	>AOW29509.1 sterol 14-demethylase [*Candida albicans* SC5314] MAIVETVIDGINYFLSLSVTQQISILLGVPFVYNLVWQYLYSLRKDRAPLVFYWIPWFGSAAS YGQQPYEFFESCRQKYGDVFSFMLLGKIMTVYLGPKGHEFVFNAKLSDVSAEDAYKHLTTP VFGKGVIYDCPNSRLMEQKKFAKFALTTDSFKRYVPKIREEILNYFVTDESFKLKEKTHGVA NVMKTQPEITIFTASRSLFGDEMRRIFDRSFAQLYSDLDKGFTPINFVFPNLPLPHYWRRDAA QKKISATYMKEIKSRRERGDIDPNRDLIDSLLIHSTYKDGVKMTDQEIANLLIGILMGGQHTS ASTSAWFLLHLGEKPHLQDVIYQEVVELLKEKGGDLNDLTYEDLQKLPSVNNTIKETLRMH MPLHSIFRKVTNPLRIPETNYIVPKGHYVLVSPGYAHTSERYFDNPEDFDPTRWDTAAAKAN SVSFNSSDEVDYGFGKVSKGVSSPYLPFGGGRHRCIGEQFAYVQLGTILTTFVYNLRWTIDG YKVPDPDYSSMVVLPTEPAEIIWEKRETCMF
Serine/ Threonine Protein kinase	>AOW28668.1 serine/threonine protein kinase [*Candida albicans* SC5314] MTSNRPPPPSLSFFIEDNPTAQQPQEHHQQSLLNPNASANRPPIKCTTTNSRFNSQSQLRYESN ARPNRSSPLFTSSPTFANYNNKPPTPSANGDDNGNSDIEDILKFPIESSHAYSYAHLSPNSLAL RLNVLKRSLEILKDRPELFKSLTTTNSATNSPVQSSAPPTATSINISIDEASPLFRSASHPVEIDT NMSTEDLSLQPPPLRKTLYMQTNHSSDYIHDVSGASSLSANEHKNYKLRSNASSAALAALF RPTMKRSDSLPLNNNTSIPVTGRRVSTPPLVRELPTSRKTSSSKNKNDDFKDIIDLLENDLSAL LDNSEVATTLHDLSLSTPDNLDDTNSNHDILKHKLLHALAMPFIENSVQPTSLLEFDGTSDPS LNSSHVRPSTTALNLLNNNYDEPPAKNRFNNNNNNNFKNSAASRPFHSLLTTKHALPQSVFT VDKDLPFSVKAANDLACLMFGVSKNTIKALTLMDLIAPQFRDFVLKRITGVQIMENKKNRD RILFAGEIVAIVRPGDQNYSWTSLWAKRKGNLIICMFDQIPCDAFDVVISSEKDVIDYKIDSIN EIAGSLIEDYGIQNLSSLNSLSYSLNKELLEFHHNNEAEQDAIDETDQTNHTNNTNRDSFDID EYYDEDIELINQTRYYTLQLEDENNVPCAITSTPLESDQEKHEIKLKIHTMPYIAGIFVIDSNE YKILSCNNAIARNLFGKSFDELENHSIDELIPNFTKILHAGIEATSNYAYQLSPGLVLPEHFFR KYDAFIKKQQNPENDQESKETIFFNSKGIEAVHRDGKVVYIDVQVRVSTNNTLVVWVTYSR TNKNRTNISEELDKLSSASTSSSISLASSNKSAVSLSSISKPRTYSSGLQLSDLKQQQQPQHSPS SSSQFLTKPSQQQSANSLQNIGGHERKLTTTSTVPSQMKLFNNEKENDLLEFSPREITRASST RKPKKETSLGIPLTRLDSYIYDKNGREQQNLQRKEEKKPAKDLQNPSSEPTLSSPLALSSQAD DSVKTDFNIVLKYTQEEILELENESLEQIKCQSSHWPKDVGTQRRTKKFSEFKVLKDMGEGA YGKVVLAQHKQDPLYKIIIKCINKERILVDTWVRDRKLGTIPSEIQIMAYLNSEPHPNIMRIID FFEDSKYYYLETPIFGDPPAIDLFDFIEIKKDLSEVESKFIFKQIVSSIYHLHKNGIVHRDIKDEN IIVDEKGVIKLIDFGSAGYVKQGPFDVFVGTIDYASPEVLGGEKYEGKPQDIWALGILLYTML YKENPFYNVDEIMEGDLRIPYVISETSLTLIKKILVRDVDERPTITDIMEDEWLQI

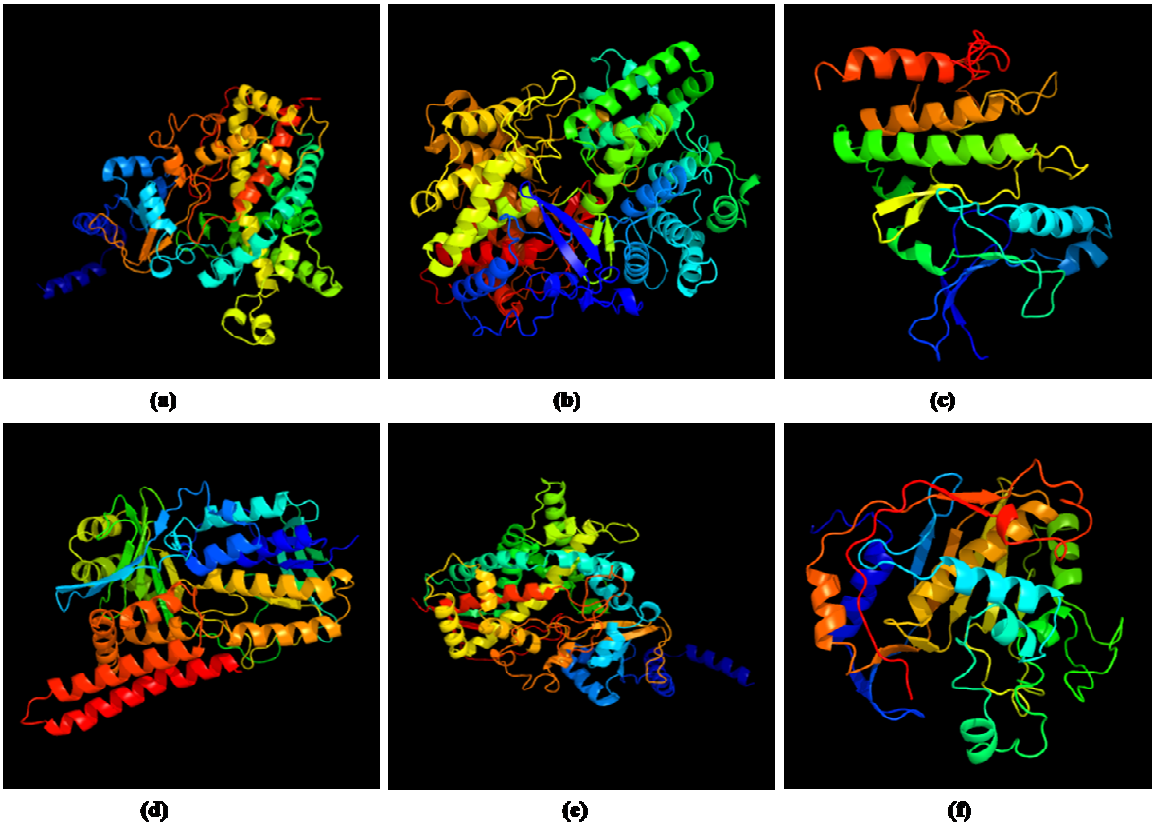

Figure 1. Structure of proteins: (a) cytochrome p450, (b) lanosterol synthase, (c) serine/ threonine protein kinase, (d) squalene monooxygenase, (e) sterol 14-demethylase, (f) thymidylate synthase.

Table 2. Confidence in the model and query coverage of the modelled structures.

Protein	Confidence (%)	Coverage (%)
Cytochrome P450	100.0	94
Lanosterol synthase	100.0	94
Serine/ Threonine protein kinase	100.0	19
Squalene monooxygenase	100.0	98
Sterol 14-demethylase	100.0	90
Thymidylate synthase	100.0	93

Table 3. Validation of the structures of modelled proteins using RAMPAGE.

Protein	Number of residues in favoured region	Number of residues in allowed region	Number of residues in outlier region
Cytochrome P450	448 (89.6%)	33 (6.6%)	19 (3.8%)
Lanosterol synthase	674 (93.1%)	31 (4.3%)	19 (2.6%)
Serine/ Threonine protein kinase	196 (76.6%)	41 (16.0%)	19 (7.4%)
Squalene monooxygenase	425 (92.8%)	25 (5.5%)	8 (1.7%)
Sterol 14-demethylase	500 (95.8%)	17 (3.3%)	5 (1.0%)
Thymidylate synthase	270 (90.9%)	17 (5.7%)	10 (3.4%)

Number of residues in the favoured region, allowed region and outlier region for each modelled structure viz. cytochrome p450, lanosterol synthase, serine/threonine protein kinase, squalene monooxygenase, sterol 14 demethylase and thymidylate synthase were determined by Ramachandran Plot (using RAMPAGE webserver) and have been mentioned in Table 3.

The structure of the ligands (ajoene, allicin, curcumin, gingerol, nimbin, nimbolide, nimonol and 6-Shogaol) obtained from PubChem have been shown in Figure 2.

Molecular docking of a particular ligand with different targets revealed that the most probable target would be the one with the highest binding energy and with the highest number of hydrogen bonds. Such targets have been tabulated with their respective binding energies in Table 4.

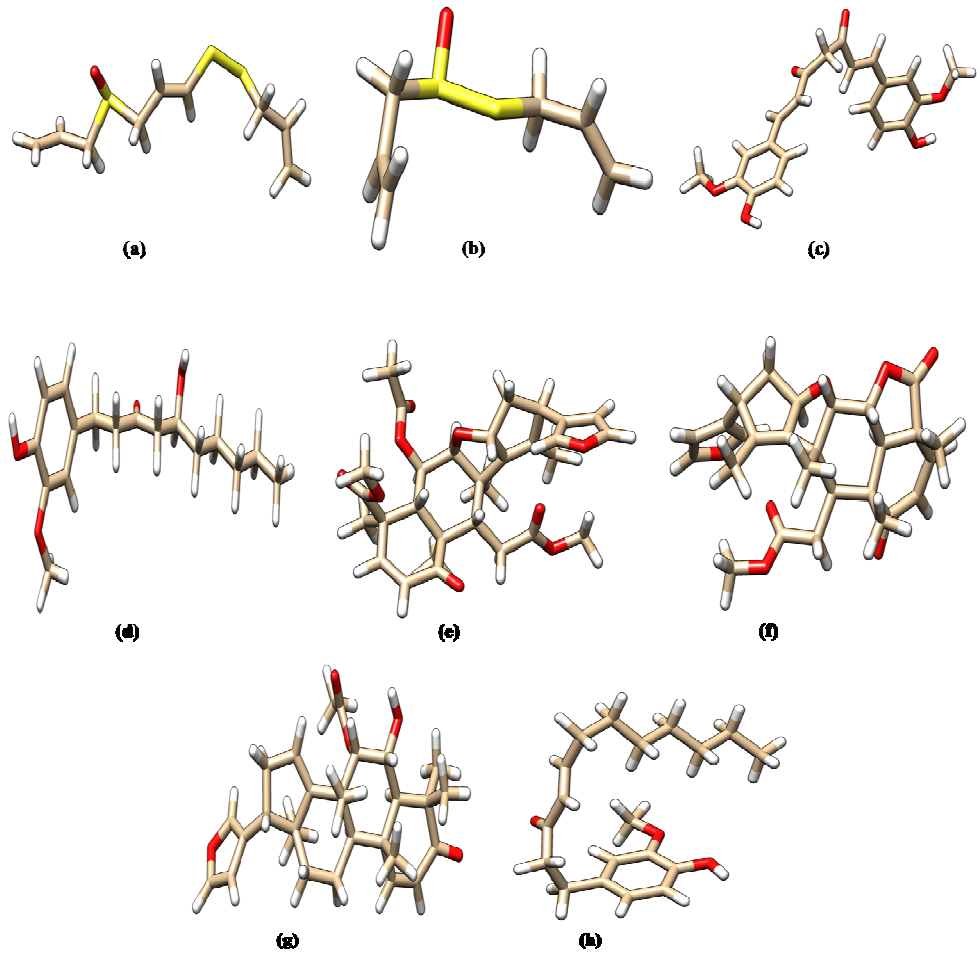

Figure 2. Structure of ligands: (a) ajoene, (b) allicin, (c) curcumin,(d) gingerol, (e) nimbin, (f) nimbolide, (g) nimonol, (h) 6-Shogaol.

Table 4. Ligands along with their most probable targets, number of hydrogen bonds and respective binding energies.

Ligand	Target	Binding energy	No. of hydrogen bonds formed
Ajoene	Sterol 14-demethylase	-43.04	0
Allicin	Cytochrome P450	-32.90	0
Curcumin	Cytochrome P450	-58.68	2
Gingerol	Cytochrome P450	-52.27	2
Nimbin	Cytochrome P450	-58.18	0
Nimbolide	Squalene monooxygenase	-60.87	2
Nimonol	Lanosterol synthase	-62.39	2
6-Shogaol	Squalene monooxygenase	-54.53	1

The respective best bind structures having the highest binding energies of the ligands docked on their targets have been shown in Figure 3.

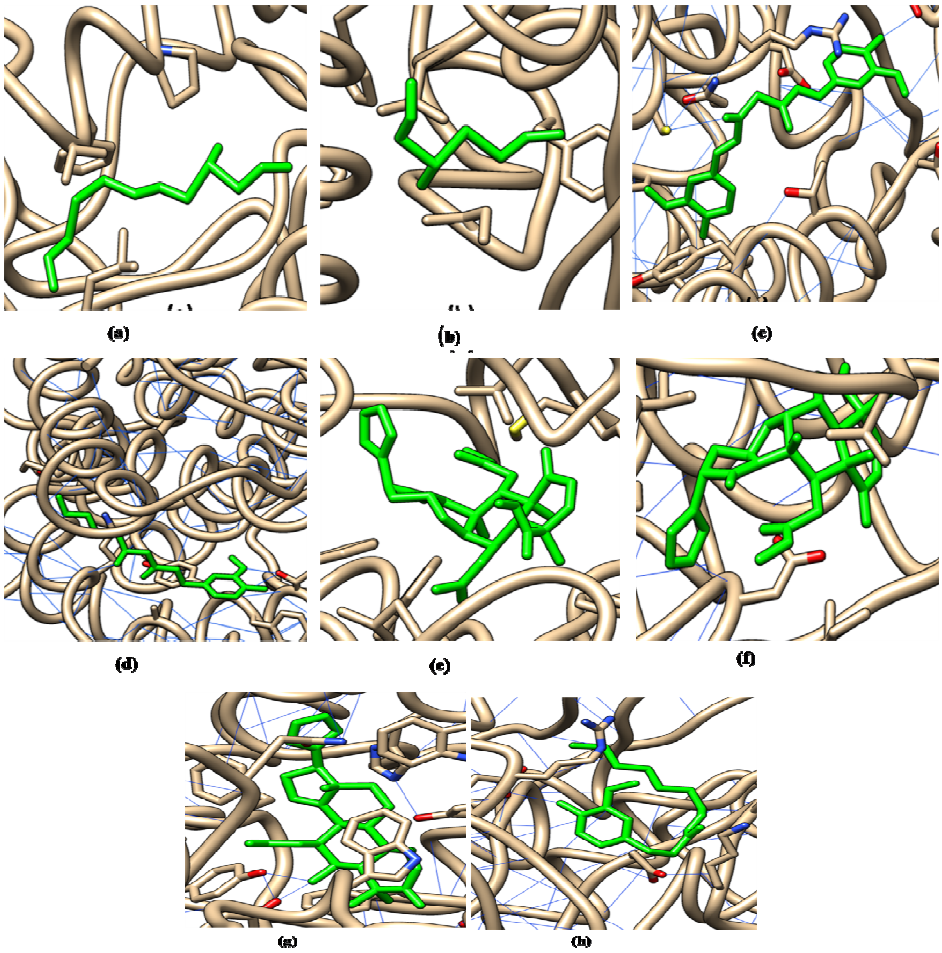

Figure 3. Target ligand complexes (a) sterol 14-demethylase vs ajoene, (b) cytochrome p450 vs allicin, (c) cytochrome p450 vs curcumin, (d) cytochrome p450 vs gingerol, (e) cytochrome p450 vs nimbin, (f) squalene monooxygenase vs nimbolide, (g) lanosterol synthase vs nimonol, (h) squalene monooxygenase vs 6-Shogaol.

4. DISCUSSION

C. albicans is the causal organism of candidiasis [17]. Recently, it has been estimated that ~700,000 cases of invasive candidiasis occurs naturally throughout the world [18]. Maximum cases of infections are caused by *C. albicans* and other *Candida* species [19]. Therefore, it becomes very essential to design a drug against *C. albicans* with minimum or no side effects. In this manuscript, we have made an effort to test some natural compounds against *C. albicans* and to determine the most probable targets of those compounds in the pathogens. The *in-silico* articles are scarce when it comes to the antifungal activity of the said ligands. However, *in-vitro* articles are available in large numbers.

According to a study in 2014, it was found that curcumin plays an important role in inhibition of *C. albicans*. The result of this study showed that the photodynamic theory can be utilized for reduction in the biofilm biomass of *C. albicans*, *C. glabrata,* and *C. tropicalis*. It was observed that the association of four LED fluences for light excitation with 40 μM concentration of curcumin at 18 J/cm^2 resulted in an inhibition of up to 85% metabolic activity of the studied *Candida* species [9]. Another study performed on curcumin

showed that the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of curcumin was found to be 800 µl and 1600 µl respectively [20]. According to a different study, it was found that curcumin modulated the proteolytic enzyme activities without down-regulating the gene expression against *C. albicans*. However, in host inflammatory responses, curcumin down-regulated IL-1β and IL-1α gene expression [21]. In 2016, a study concluded that curcumin (5-20 mM) inhibited *C. albicans* morphogenesis significantly and also exhibited considerable activity against biofilm formation [22].

According to Mahmoud et al. [6], neem contains nimonol that gives good response against *C. albicans*. Different types of extracts from neem leaves were found to have inhibitory effect on *C. albicans*. Neem is the natural compound which contains nimbin, nimbolide, nimonol etc. that bind to their respective targets and inhibit the growth of *C. albicans* [6]. Santos et al., determined the minimum inhibitory concentration (MIC) of Nimonol against of *C. albicans* to be 1000/500 µg//mL [23].

Yoshida et al. [8], showed that at less than 20 µg/ml of ajoene, growth of *C. albicans* was inhibited [8]. Similarly, allicin present in garlic was also found to have the ability to inhibit growth of *C. albicans* and reduce the MIC of amphotericin B while keeping the same efficacy [7]. In a different study allicin was found to contain allyl sulphur derivative of garlic. The MIC50 and MIC90 of allicin alone against six *Candida* spp. ranged from 0.05 to 25 µg/ml [24]. Bansal and co-workers in 2019 [25] showed that MIC of neem extract was 5.0 mg/ml against *C. albicans* and antifungal activity was found to be highest for chlorhexidine (14.4 Mm) followed by neem [25].

In ginger, 6-gingerol and 6-Shogaol showed antifungal response against *C. albicans*. Lee et al. [10], showed that 6-gingerol and 6-shagaol had the intrinsic potential to reduce the *C. albicans* virulence in a nematode infection model. They also emphasized upon the antibiofilm and antivirulence activities of these compounds against drug resistant *C. albicans* [10]. According to Kandhan et al., the ethanolic extract of *Z. officinale* showed good antifungal activity at different concentrations with a maximum zone of inhibition of 21 mm at concentration of 2000 µg/ml [26].

5. CONCLUSION

The current manuscript focuses upon prediction of the antifungal targets of natural compounds (allicin, ajoene, curcumin, gingerol, nimbin, nimbolide, nimonol and 6-shogaol) against *C. albicans.* In conclusion, the best suited target for ajoene, allicin, curcumin, gingerol, nimbin, nimbolide, nimonol and 6-shogaol are sterol 14-demethylase, cytochrome p450, cytochrome p450, cytochrome p450, cytochrome p450, squalene monooxygenase, lanosterol synthase and squalene monooxygenase, respectively. Although, these compounds have been tested *in-silico* but determination of their *in-vitro* and *in-vivo* antifungal activity is desirous.

Authors' Contributions: Both authors contributed equally to this work. Both authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

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