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In silico anti-fungal efficacy and the mechanism of binding of some *Syzygium aromaticum* ingredient compounds to aspartate semialdehyde dehydrogenase, 6C8W and 6C85, enzymes from *Blastomyces dermatitidis*

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Abstract: This in silico work was carried out to reveal the proposed anti-fungal efficacy of some clove ingredient compounds against aspartate semialdehyde dehydrogenase, 6C8W and 6C85, enzymes from *Blastomyces dermatitidis*. The molecular docking simulation was implemented utilizing the Auto Dock 4.2. software. A set of 17 compounds were selected for this study, which is known to be active ingredients of *Syzygium aromaticum* crude and oil. The best docking scores associated with the *Blastomyces dermatitidis* enzymes 6C85 and 6C8W were for Maslinic acid and Oleanolic acid, followed by Stigmasterol and Campesterol. It was found that these compounds possess inhibitory potential against 6C85 and 6C8W and hence have anti-fungal efficacy. Maslinic acid and Oleanolic acid produced the strongest binding to 6C85 and 6C8W over the remaining bioactive compounds by forming H-bonds with some amino acids in these enzymes.

Keywords: *Blastomyces dermatitidis, Syzygium aromaticum,* aspartate semialdehyde dehydrogenase, maslinic acid, Oleanolic acid.

INTRODUCTION

The explore for new medicines extracted from plants has quickened for the time being. Microbiologists, phytochemists and botanists are sieving the world for phytochemical ingredients which might be improved for treating infections (Mohammadi *et al.*, 2014). The optionality for the treatment of fungal diseases is extremely limited when matched with those available antibacterial drugs. Only three groups of anti-fungal drugs are presently utilized in clinical practice and just one newfangled group of anti-fungal medications has been released within the last 3 decades. Hence, there is a stately challenge for novel anti-fungal drug discovery (Roemer and Krysan, 2014). Moreover, recognizing novel drug targets is challenging because there are many resemblances between fungal and human cells (Scorzoni *et al.*, 2017).

Herbal medicinal products are authenticated as a significant exporter for disclosing recent pharmaceutical compounds that are utilized to handle dangerous diseases. Plentiful herbs have been declared to possess pharmacological potentials owing to their phytoconstituents such as terpenes, glycosides, steroids, flavonoids, tannins, alkaloids and many others. Numerous plant extracts exhibit significant antimicrobial activity and can be used therapeutically (Shalayel *et al.*, 2017). Clove, *Syzygium aromaticum*, is a traditional condiment with multiple pharmacological efficacies and has typically been utilized as a preservative (Batiha *et al.*, 2020). Cloves are used as a carminative, to improve peristalis and to boost gastric acid. In addition to that, Cloves have antioxidant, antimutagenic, antiulcerogenic, anti-inflammatory, antithrombotic and antiparasitic properties, as well as antibacterial and anti-infective properties (Pandey and Singh, 2011).

Alpha-pinene, furfural, and methyl salicylate are being found in the oil (Elkins, 1979). Sixteen volatile compounds were identified in an n-hexane extract of *Syzygium aromaticum* buds by using GC-MS. The main components were eugenol (71.56%) and eugenol acetate (8.99%). (Nassar *et al.,* 2007). Confirmed 3-allyl-6-methoxyphenol at a concentration of 69.44% in another study (Azir Uddin *et al.,* 2017). Close to 12-26% of the oil is eugenol, with beta-caryophyllene accounting for 70-90% of it (as large as 7 percent). Also, other constituents have been reported in Clove. Acetyl eugenol, beta-caryophyllene, vanillin and crategolic acid are all found in these component ingredients (Batiha *et al.*, 2020, Mittal *et al.*, 2014). in certain experiments, volatile vapour from clove essential oil had fungistatic effects, while applied directly, it possessed fungicidal properties (Chee and Lee, 2007). Wankhede (2015) claimed that *Syzygium aromaticum* has a synergistic interaction against microbial

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infections and can be developed for therapeutic purposes (Wankhede, 2015).

The ascomycetous fungus *Ajellomyces dermatitidis* is referred to as *Blastomyces dermatitidis* (Salares 2007). By 2013, a second species in the genus Blastomyces, *B. gilchristii*, had been identified, encompassing several strains previously allocated to *B. dermatitidis*. *Blastomyces emzantsi*, *Blastomyces parvus* and *Blastomyces percursus* are three more species that have been discovered (Brown *et al.*, 2016). Blastomycosis is caused by the fungus *Blastomyces dermatitidis*, which causes a severe fungal infection in humans and animals in certain endemic areas (DiSalvo, 1992).

Inhalation of bacterial spores of *Blastomyces dermatitidis* present in the environment causes blastomycosis, a potentially fatal mycosis. This fungus' ecological pattern is not well known (Reed *et al.*, 2008). Previous studies have linked blastomycosis with particular soil chemicals. Blastomycosis is potentially affecting urban and suburban territories (Huber *et al.*, 2016).

This study aimed to show the proposed in silico anti-fungal efficacy and the mechanism of binding of some clove ingredient compounds to aspartate semialdehyde dehydrogenase, 6C8W and 6C85, enzymes from Blastomyces dermatitidis.

MATERIALS AND METHODS

Molecular docking simulation were used in order to discover the better realize and explained how the chemical groups within ligand that can be found in clove might have got influenced their routines. The molecular docking simulation is transported out utilizing the AutoDock 4.2. software. Molecular docking was applied on 6C8W, *Blastomyces dermatitidis* aspartate semialdehyde dehydrogenase crystal form with NADP and 6C85, crystal structure of aspartate semialdehyde dehydrogenase from *Blastomyces dermatitidis* with p-benzoquinone. These particular crystal structures are usually downloaded in the PDB (the protein data bank) format file from the PDB as a data source for structure-based design purposes.

The protein preparation protocol was performed to prepare the particular enzymes using BIOVIA Discovery Studio Visualizer 16. 1. The particular 2D structure associated with ligand built making use of Chem Draw software sixteen. 0. 0. 82 and input to energy minimization. Lone pairs and non-polar hydrogens will be then integrated. Also each atom was designed with Gasteiger partial charges (Rizvi *et al.*, 2013).

The final results associated with the docking had been saved in pdb file format. The gained log data files were adopted in Auto Dock Equipment (ADT) to analyze the output results of docking and find out the inhibitory constant (Ki) and the binding power (BE) for every ligand with the target protein. The creation of the structural complex of the particular ligands with proteins was analyzed along with the help of BIOVIA Discovery Studio Visualizer 16.1 (Sadati *et al.*, 2019).

A set of 17 compounds were selected for this study, which is known to be active ingredients of *Syzygium aromaticum* crude and oil, including β-caryophyllene, Maslinic acid, Bicornin, Methyl salicylate, Tannic acid, Eugenin, Kaempferol, Eugenitin, Vanillin, Rhamnetin, Terpenoid (taxol), Oleanolic acid, Campesterol, Stigmasterol, Zingiberene, δ-Cadinene and Humulone (Batiha *et al.*, 2020, Mbaveng and Kuete, 2017, Mittal *et al.*, 2014). All selected compounds were downloaded from the Pub Chem Database. Finally, Perkin Elmer Chem3D 17.1 software was used to enforce MM2 force field to the compounds and all saved as PDB format.

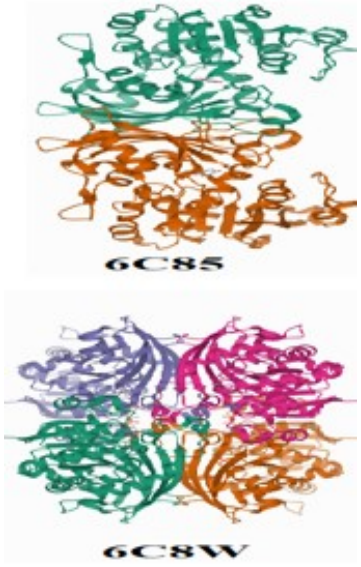
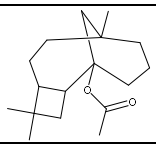
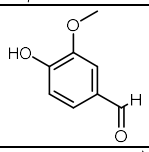
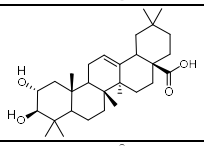
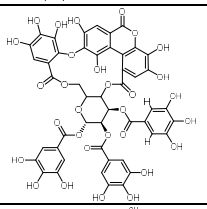
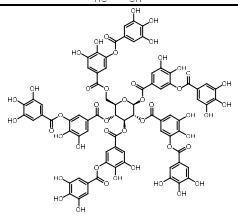
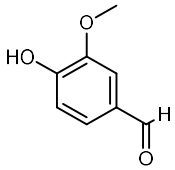
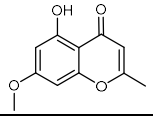
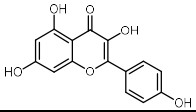
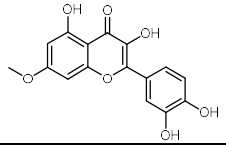
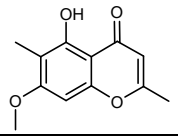


Fig. 1: Crystal structures of aspartate semialdehyde dehydrogenase from *Blastomyces dermatitidis* with p-benzoquinone, 6C85 and Aspartate Semialdehyde Dehydrogenase with NADP from *Blastomyces dermatitidis*, 6C8W (Adapted from https://www.rcsb.org/structure/6C85, https://www.rcsb.org/structure/6C8W)

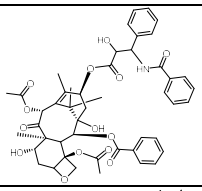
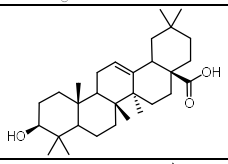
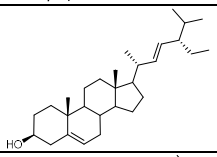
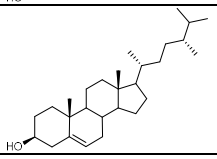
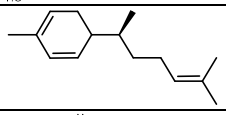
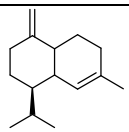
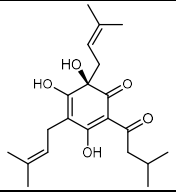
Active Site Prediction

All possible binding sites of 6C8Wand 6C85 were searched by active analysis Prank Web server (http://prankweb.cz). The preparation of aspartate semialdehyde dehydrogenase enzyme target molecules were selected for the docking study. The crystal structures of the enzyme are available in Protein Data Bank (PDB, http:/www.rcsb.org/pdb) and the PDB ID for these structures (6C85, 6C8W) has been optimized and processed for molecular docking simulation.

Table 1: Molecular docking scores of the bioactive isolated compounds of *Syzygium aromaticum* and Inhibition Constant (Ki in micromolar) to *Blastomyces dermatitidis* (6C85, 6C8W)

Compounds	2D-Structure	Docking Score Kcal/Mol (6C85)	Ki uM	Docking Score Kcal/Mol (6C8W)	Ki uM
B-caryophyllene		-6.82	10.08	-7.61	2.630
Vanillin		-4.56	456.80	-4.35	589.20
Maslinic acid		**-9.71	0.076	**-10.06	0.043
Bicornin		-3.72	1000.86	-4.22	604.05
Tannic acid		++++	--------	++++	-------
Methyl salicylate		-5.28	135.04	-4.48	521.24
Eugenin		-6.35	22.03	-5.31	127.13
Kaempferol		-6.44	22.98	-6.40	20.34
Rhamnetin		-6.83	9.91	-6.86	9.33
Eugenitin		-5.77	51.99	-5.73	63.27

Continue…

Terpenoid (taxol)		-5.84	52.76	-8.04	1.27
Oleanolic acid		**-9.53	0.103	**-9.90	0.055
Stigmasterol		*-8.10	1.16	*-8.92	0.291
Campesterol		*-8.19	0.986	*-8.97	0.264
Zingiberene		-5.57	82.34	-5.76	59.50
δ-Cadinene		-6.12	32.88	-5.99	40.42
Humulone		-7.58	2.76	-7.46	3.55

Significant results are denoted by * and **

Protein Preparation

The studied fungi proteins crystal structures have been downloaded from PDB (Patrick, Nakatani *et al.*, 2010). The heteroatoms and water were discarded by Biovia Discovery Studio Visualizer 16.1.

Molecular Docking software

This part was achieved by using Auto Dock 4.2 software, where all rotatable bonds of the compounds were set randomized as completely flexible during the simulation process. The 2D and 3D potential were visualized and analyzed by the Discovery Studio Visualizer 16.1, to be able to easily observe the hydrogen bonds and the hydrophobic interactions. The Genetic Algorithm (GA) run number was set to 150 runs and the Lamarckian algorithm was used to simulate this process. Docking scores were interpreted using Discovery Studio Visualizer 16.1 and Ligand Scout 4.3 academic license, so that ionic bonds, hydrogen bonds and hydrophobic interactions could be easily observed.

STATISTICAL ANALYSIS

Auto Dock version 4.2 was used to simulate the docking process. In addition, Discovery Studio Visualizer 16.1 and Ligand Scout 4.3 was used to show the docked visualization analysis.

RESULTS

Table 1 displays the computed scores of dockings between the two aspartate semialdehyde dehydrogenase structures, fig. 1 and the bioactive compounds. The low energy value demonstrates the probability of highly compound interaction while the high docking value percolates to be a possible weak relation with the enzyme. The selected bioactive compounds showed varying scores to the two crystal structures of the enzyme. The best biological compounds associated with the *Blastomyces dermatitidis* enzymes 6C85 and 6C8W were Maslinic acid and Oleanolic acid, followed by Stigmasterol and Campesterol.

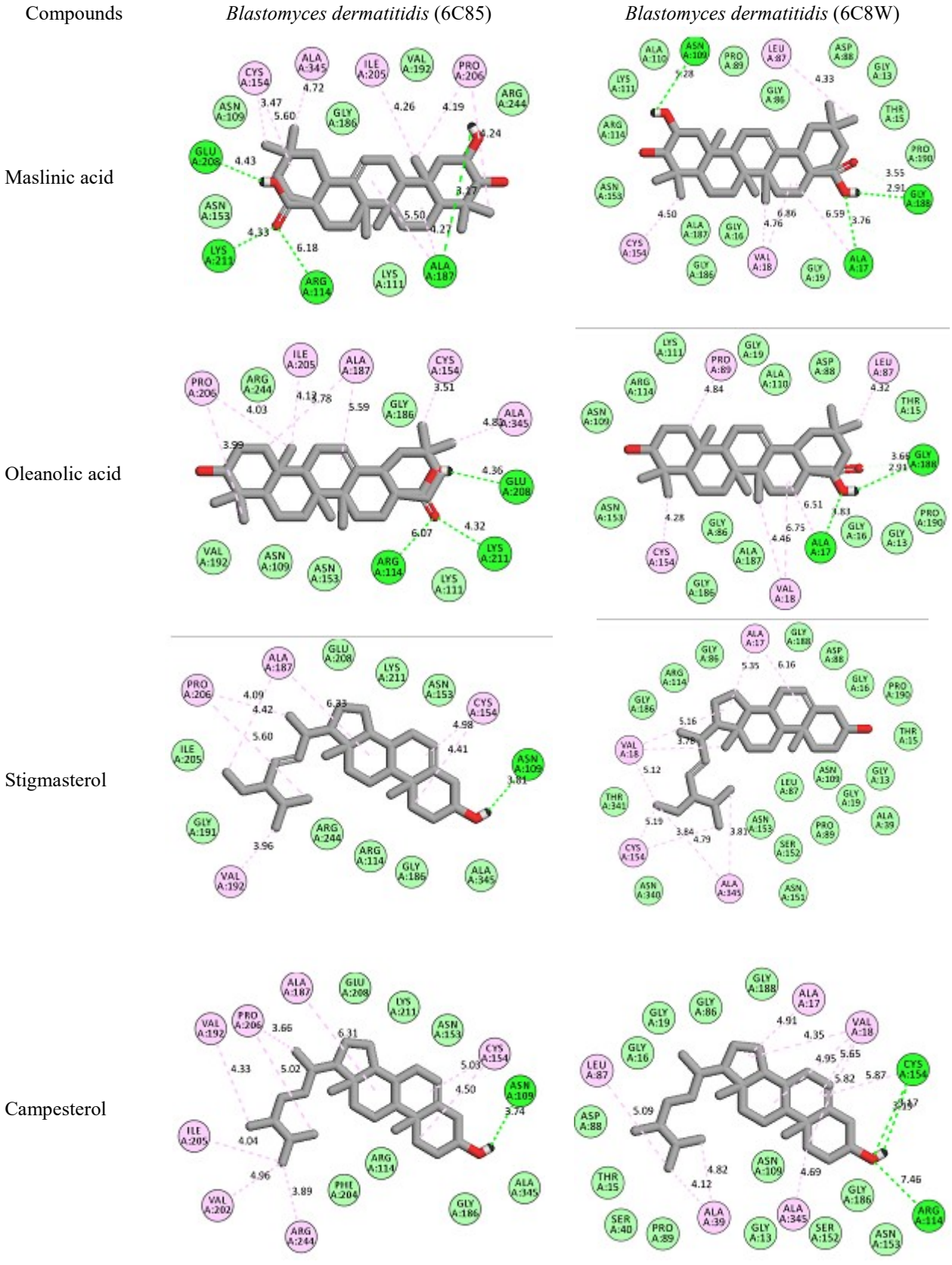


Fig. 2: 2D interactions for the best bioactive compounds scores using Discovery Studio Visualizer 16.1

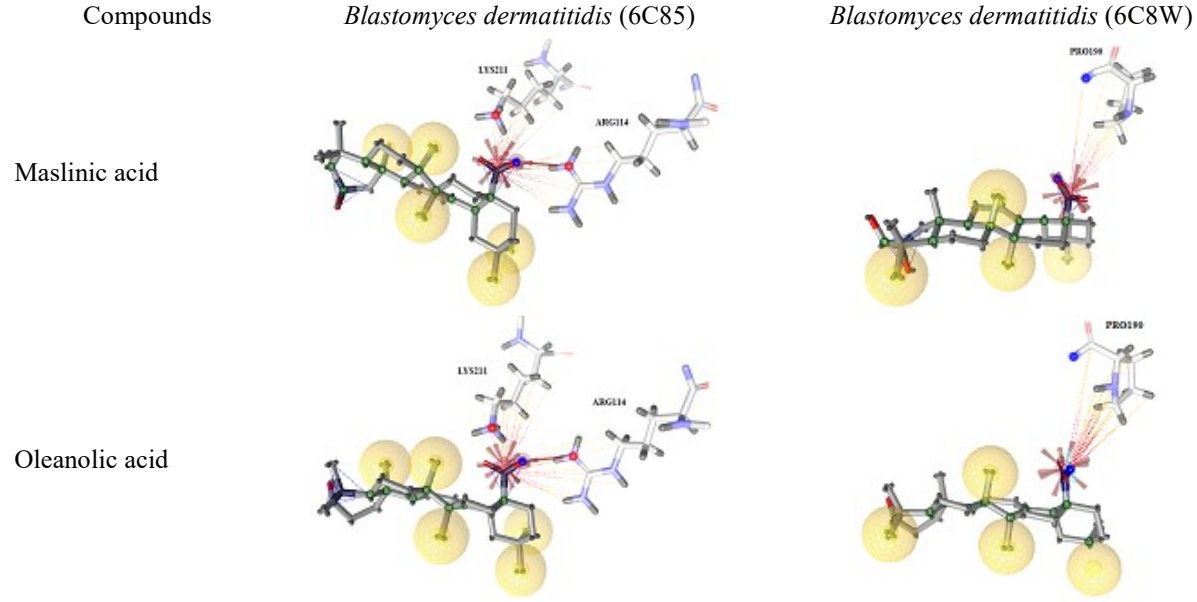


Fig. 3: Ligand Scout pharmacophore models to PDB (6C85, 6C8W) complexes with the ligands (Maslinic acid and Oleanolic acid).

The 2D interactions of the best complexes have been displayed in fig. 2. Maslinic acid and Oleanolic acid were lowest energy values for Aspartate Semialdehyde Dehydrogenase with binding energy to 6C85 -9.71 and -9.53 and docking energy to 6C8W -10.06 and -9.90 Kcal/ mol, respectively.

The carboxylic acid group of Maslinic acid and Oleanolic acid have played a key role in the binding over the remaining bioactive compounds by forming H-bonds with ARG114, GLU208 and LYS211 from the crystal structure of 6C85 as well as ALA17 and Gly188 with 6C8W as shown in fig. 2. Amazingly, in structure 6C85, the guanidino group of ARG114 and the amino group of LYS211 formed a very strong interaction (ionic interaction bond) with the carboxylic acid group of Maslinic acid and Oleanolic acid, which led to an improvement in the affinity of the inner pocket interactions. By the same token, the carboxylic acid group of Maslinic acid and Oleanolic acid have formed Ionic interaction with the nitrogen atom of pyrrolidone ring from PRO190 in 6C8W, as shown in fig. 3.

DISCUSSION

Comprehensive notarization of the antimicrobial features for the constituents of essential oils has been accomplished by various researchers. While the mechanism of action of a few essential oil components has been explained in several leading previous studies, thorough understanding of the majority of the compounds and their mechanisms of action remains missing. It necessitates the creation of new anti-fungal compounds to treat fungal infections (Pandey and Singh, 2011) especially when we put into consideration the costiveness and the adverse effects of the already utilized anti-fungal therapeutics.

Molecular docking studies in the antifungal efficacy of clove oil ingredients are scarce and as far as we know, no previous studies reported the nature of inhibition excreted by clove oil ingredients against some key enzymes in fungi. However, some recent studies evaluated the binding characteristics of some previously reported compounds from clove to some key metabolic regulators in cancer (Gyebi *et al.*, 2019).

The molecular docking and computational works were used to demonstrate the anti-fungal efficacy of the most abundant active ingredients of clove oil against *Blastomyces dermatitidis* (Shalayel *et al.*, 2021, Shalayel *et al.*, 2021). Our results revealed many potent components in clove oil like Maslinic acid, Oleanolic acid, Stigmasterol and Campesterol with anti-fungal potency against Aspartate semialdehyde dehydrogenases (6C85, 6C8W) in *Blastomyces dermatitidis*.

Fungi and other bacteria use aspartate semialdehyde dehydrogenase to catalyse the first branch step of the anabolic pathway, as well as the higher plants utilizing aspartate to produce amino acids like methionine and lysine and diaminopimelate is a part of the cell wall. Since inhibition of this biosynthetic pathway, which is not included in humans, is lethal, inhibitors of this enzyme may be an effective antibacterial, fungicidal, or herbicidal agent (Hadfield *et al.,* 2001). Furthermore, a recent study demonstrated that Aspartate semialdehyde dehydrogenase enzyme is a promising novel agent for further progress as

a new anti-fungal medication against *C. healbicans* and some other fungal components (Dahal *et al.*, 2020).

Various fungal aspartate semialdehyde dehydrogenase structures were specified. However, the previous crystallisation parameters had hampered the formation of complexes with enzyme suppressors. The pathogenic fungi's first inhibitor-bound and cofactor-bound forms of these enzymes (6C85, 6C8W) and a structural and functional link to the other aspartate semialdehyde dehydrogenase family members have now been determined in *blastomyces dermatitidis* (Dahal and Viola, 2018) like 4ZIC, the crystal form of aspartate semialdehyde dehydrogenase with NADP, which is specific for *Trichophyton spp*. especially *T. rubrum*.

In the present study, the carboxylic acid group of Maslinic acid and Oleanolic acid have played a key role in the binding over than the remaining bioactive compounds by forming H-bonds with ARG114, GLU208 and LYS211 from the crystal structure of 6C85 as well as ALA17 and Gly188 with 6C8W. In 6C85 structure, the guanidino group of ARG114 and the amino group of LYS211 formed a very strong interaction (Ionic interaction bond) with the carboxylic acid group of Maslinic acid and Oleanolic acid, which led to an improvement in the affinity of the inner pocket interactions. By the same token, the carboxylic acid group of Maslinic acid and Oleanolic acid have formed Ionic interaction with the nitrogen atom of pyrrolidone ring from PRO190 in the crystal structure 6C8W.

The finding that Stigmasterol and Campesterol potentially inhibited the active key enzymes 6C85 and 6C8W of *Blastomyces dermatitidis*, may be explained by the fact that these sterols may exert competitive inhibition in displacement of ergosterol component of the fungal membrane. The azole anti-fungal group functions by blocking lanosterol 14-alpha-demethylase, a fungus cellular membrane enzyme that transforms lanosterol to ergosterol (Zonios and Bennett, 2008). The capacity of certain active components of clove to create complexes with ergosterol was investigated in order to evaluate their effects on the fungal cell wall (ergosterol effect assay) (Martinez-Rossi *et al.*, 2008). The anti-fungal action of clove oil components can be attributed in part to their hydrophobic and lipophilic properties, which enable them to divide into the lipid bilayer, causing permeability to change and consequent leakage of cellular membrane (Burt, 2004). Scanning electron microscopy and transmission microscopy of *C. gloeosporioides* exposed to clove oil revealed significant ultra structural and morphological alterations focusing the disruption of the fungal cell wall and endomembrane network, resulting in increased permeability as well as the loss of intracellular constituents (Zhang *et al.*, 2019).

CONCLUSION

To the best of our knowledge, this is the first study to use molecular docking and computational methods to show that clove oil's active components have an anti-fungal effect against *Blastomyces dermatitidis*. Oleanolic acid, Maslinic acid, Stigmasterol and Campesterol are active components in clove oil with anti-fungal activity against aspartate semialdehyde dehydrogenases (6C85, 6C8W) in *Blastomyces dermatitidis*. These two compounds (Maslinic acid and Oleanolic acid) bound more strongly to 6C85 and 6C8W by creating H-bonds with amino acids in these enzymes. Based on our results, possible in vivo trials may begin for clove crude and oil components to treat blastomycosis and other fungal illnesses.

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