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# Molecular Docking Evaluation of *Syzygium aromaticum* Isolated Compounds Against Exo-β-(1,3)-glucanases of *Candida albicans*

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors MHFS, SN and GMAM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FKAS and SA managed the analyses of the study. Authors ATA and AM managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

Seventeen compounds from *Syzygium aromaticum* are selected for exo-β-(1,3)-glucanases inhibitory activity by using molecular docking study. The compounds are uploaded from the PubChem database and molecular docking with AutoDock 1.5.6 tools is carried out. The molecular docking scores indicate that stigmasterol and campesterol are of the highest potentials, and approximately have similar binding affinities with *Candida albicans*' active site (3N9K, 3O6A). The hydroxyl moiety has played an important role in the antifungal potentiality of all studied compounds.

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*Keywords:* *Candida albicans*; *syzygium aromaticum*; exo-β-(1,3)-glucanases; stigmasterol; campesterol.

## 1. INTRODUCTION

*Candida albicans* is responsible for about 70 – 80% of all candidal infections. Other significant species include *C. glabrata, C. tropicalis, C. krusei, and C. dubliniensis*. Candidiasis occurs most commonly in intertriginous areas such as the axillae, groin, and gluteal folds (e.g., diaper rash), in digital web spaces (interdigital space), on the glans penis, and beneath the breasts. Vulvovaginal candidiasis is common among women. *Candidal* nail infections may develop after improperly done manicures and in kitchen workers and others whose hands are continually exposed to water (Onychomycosis) [1,2]. *Candida albicans has the ability to endure* multiple morphological transitions to infect an enormous diversity of hosts [3]. Hyphal formation is a fundamental issue for diverse hosts such as virulence and invasion. For this purpose, many environmental stimuli can stimulate *Candida albicans* to compose hyphae like quorum sensing molecules, carbon dioxide ($CO_2$), and the negative log of the hydrogen ion concentration (pH) of the environment [4]. Exo-β-(1,3)-glucanases are group of fungal enzymes including that from the human pathogen *Candida albicans and* belong to family 5 glycosyl hydrolase. They possess both hydrolase and transferase leverage functioning in cell wall morphogenesis and glucan metabolism [5].

Recently, computational research methods have turn into a crucial component in the drug discovery process. Molecular modelling is among the strategies mainly used as strike recognition tool when the 3D–crystal structure of the macromolecules and chemical structure of the ligands are known [6]. Docking method is an energy-based calculation process that determines the most spontaneous binding of the ligand conformation with the active site of the target. The lowest energy values are usually considered optimal for the protein-ligand complexes compared to the highest energy scores. Therefore, molecular docking can be used as an optimization method, used to point out the lowest energy ligand binding with the target [7]. A huge number of molecular docking programs, like AutoDock, MOE, Molegro Virtual Docker and Hex, have been used in the last ten years ago to simulate the docking process [8]. We have selected AutoDock 1.5.6 tools for this work. This study aimed to reveal the proposed inhibitory activity of some clove ingredient compounds to 3N9K, the crystal structure of F229A/E292S Double Mutant of Exo-Beta-1,3-Glucanase and 3O6A, the crystal structure of F144Y/F258Y Double Mutant of Exo-beta-1,3-glucanase from *Candida albicans*.

## 2. EXPERIMENTAL PROTOCOLS

### 2.1 Ligand Preparation

A set of 17 compounds had been selected for this study, which is isolated from *Syzygium aromaticum* crude and its oil as β-caryophyllene, Maslinic acid, Bicornin, Methyl salicylate, Eugenin, Tannic acid, Kaempferol, Vanillin, Rhamnetin, Eugenitin, Terpenoid (taxol), Oleanolic acid, Stigmasterol, Campesterol, Zingiberene, δ-Cadinene, and Humulone. The highest concentration percentages were for Eugenin and β-caryophyllene [9-11]. All selected compounds are downloaded from the PubChem Database. Finally, PerkinElmer Chem3D 17.1 software is used to enforce MM2 force field to the compounds and all are saved as PDB format.

### 2.2 Active Site Prediction

We study the whole possible binding sites of *Candida albicans* (3N9K, and 3O6A) by using the active analysis PrankWeb server (http://prankweb.cz/). We find that there are six and five possible binding sites in 3N9K and 3O6A, respectively. Accordingly, we select the largest site with the highest pocket score of the corresponding *Candida albicans*. Further, our selection of the binding site depends on the fact of Laminaritriose bonding to that site, and the selected pocket score grid center (X, Y, and Z) of 3N9K and 3O6A are (-3.1917, -7.5161, 10.9316) and (3.2975, -7.4503, -10.7674), respectively.

### 2.3 Protein Preparation

The *Candida albicans* proteins are downloaded from the protein data bank database (www.rcsb.org/pdb) (PDB ID: 3N9K, and 3O6A) [12]. The heteroatoms and water are discarded by Biovia Discovery Studio Visualizer 16.1.15350.

## 2.4 Molecular Docking

This part is achieved by using AutoDock 4.2 software, where all rotatable bonds of the compounds are set randomized as completely flexible during the simulation process. A maximum number of 100 runs are chosen for each independent Lamarckian genetic algorithm. The 2D and 3D potential are visualized and analyzed by the Discovery Studio Visualizer 16.1, to be able easily observed the hydrogen bonds and the hydrophobic interactions.

## 3. RESULTS AND DISCUSSION

Table 1 shows the calculated scores of the possible interactions between the targeted *Candida albicans* and the selected compounds. The low energy score showed the possibility of highly compound interaction, whereas the high docking value reverberates possible weak binding with the targeted *Candida albicans.*

The lower docking score with lower compound inhibition constant means stronger drug molecule binding to the active site and more potent. The order of the stability (the most to the least stable) of *Candida albicans* Exo-β-(1,3)-glucanases (3N9K and 3O6A) is given in Table 2 as follows: Stigmasterol, Campesterol complexes, followed by Oleanolic acid, Bicornin, Terpenoid (taxol), Humulone, Maslinic acid, and finally B-caryophyllene. Table 2 shows that the whole selected compounds are positioned in the binding groove of the enzyme that favors hydrophobic interactions with both binding sites of amino acids residues.

The binding interactions in the binding site of Stigmasterol and Campesterol are the same as those of Oleanolic acid, Bicornin, Terpenoid (taxol), Humulone, Maslinic acid, and B-caryophyllene (3N9 K, 3O6A). This can be attributed to the H-bonding interactions with different types of amino acids at the binding sites [13]. Consequently, the hydroxyl moiety in all selected compounds is responsible for that stability, Table 2.

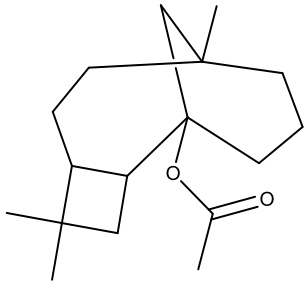
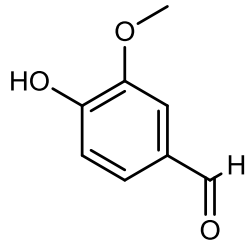
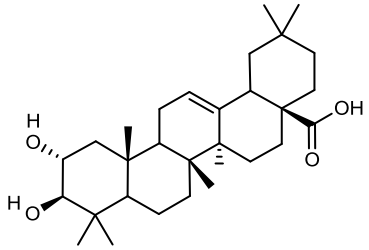
Fungal resistance to antibiotics may be justified by the fact that the fungal cells are equipped with a detoxifying system, which is able to modify many antibiotics, probably by hydroxylation [14]. Hence, the antibiotics utilized to treat the fungal infection will retain fungistatic for a period of time, and recurrent usage of such antibiotics is highly recommended. The effective antifungal drugs may extract or modify membrane sterols or prevent their synthesis [15].

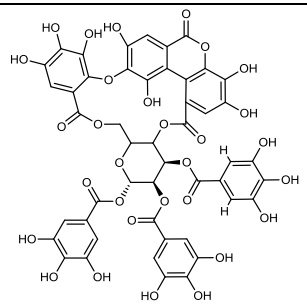
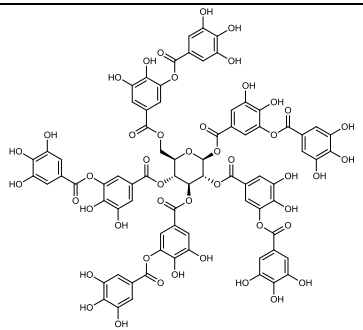
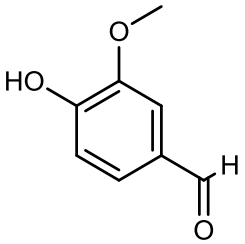
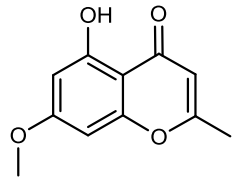
Some active ingredients in many medicinal herbs including clove (Syzigium aromaticum), like eugenol, exert their antifungal effects on the cell wall and cell membrane of fungi. Eugenol acts on cell membrane by a mechanism that seems to involve the suppression of ergosterol biosynthesis. The lower ergosterol content interferes with the integrity and functionality of the cell membrane [16]. *Candida albicans* has the ability to endure multiple morphological transitions to infect an enormous diversity of hosts [17]. For virulence and invasion in those diverse hosts, hyphal formation is a fundamental issue. For this purpose, many environmental stimuli can stimulate *Candida albicans* to compose hyphae like quorum sensing molecules, $CO_2$, and pH of the environment [18,19].

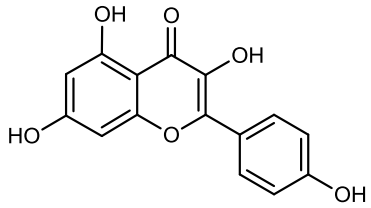
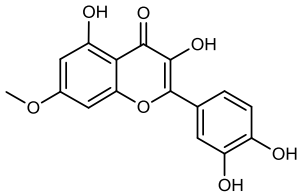
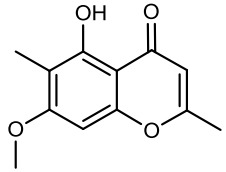
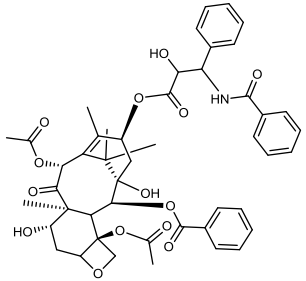
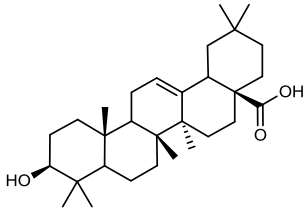
Exo-β-(1,3)-glucanases are group of fungal enzymes including that from the human pathogen *Candida albicans* and belong to family 5 glycosyl hydrolase. They possess both hydrolase and transferase leverage functioning in cell wall morphogenesis and glucan metabolism [20].

From the obtained results that discussed above one can conclude that Stigmasterol and Campesterol can effectively inhibit both *candidal* exo-β-(1,3)-glucanases (3N9K and 3O6A). Their association with commercial antifungal therapeutics used in candidiasis treatment, possibly will bring benefits based on the striking effect on germ tube formation [21]. The finding that Stigmasterol and Campesterol potentially inhibited the active key enzymes 3N9K and 3O6A of *Candida albicans* may be explained by the fact that these sterols may exert competitive inhibition in displacement of ergosterol component of the fungal membrane. The azole antifungal family works by inhibiting lanosterol 14-alpha-demethylase, which converts lanosterol to ergosterol in fungus cellular membranes [22]. The ability of some other active components of clove to form complexes with ergosterol is evaluated from the perspective of investigating their actions on the fungal cell membrane (ergosterol effect assay) [23].

**Table 1. Compounds of *Syzygium aromaticum* with autodock4.2 score, and inhibition constant ki to *Candida albicans* (3N9K, 3O6A).**

| Compounds | 2D-Structure | *Candida albicans* (3N9K) AutoDock Score Kcal/Mol | Inhibition Constant KiuM (micromolar) | *Candida albicans* (3O6A) AutoDock Score Kcal/Mol | Inhibition Constant KiuM (micromolar) |
|---|---|---|---|---|---|
| B-caryophyllene |  | -9.20 | 0.180 | -8.16 | 1.05 |
| Vanillin |  | -5.87 | 49.64 | -5.06 | 196.3 |
| Maslinic acid |  | -9.20 | 0.180 | -8.37 | 0.735 |

| | | | | | |
|---|---|---|---|---|---|
| Bicornin |  | -9.48 | 0.113 | -10.07 | 0.041 |
| Tannic acid |  | +131.0 | ------ | +144.5 | ------ |
| Methyl salicylate |  | -5.86 | 50.59 | -5.05 | 197.3 |
| Eugenin |  | -6.82 | 10.34 | -6.00 | 40.22 |

| | | | | | |
|---|---|---|---|---|---|
| Kaempferol |  | -8.07 | 1.21 | -8.67 | 0.443 |
| Rhamnetin |  | -8.16 | 1.05 | -8.17 | 1.02 |
| Eugenitin |  | -6.57 | 15.34 | -6.15 | 31.25 |
| Terpenoid (taxol) |  | -9.16 | 0.192 | -9.85 | 0.060 |
| Oleanolic acid |  | -10.25 | 0.030 | -9.85 | 0.353 |

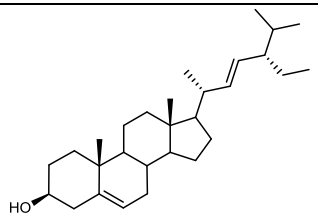
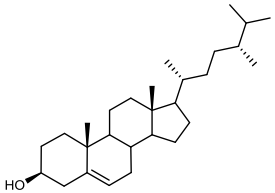
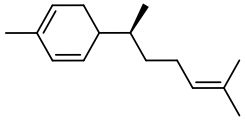
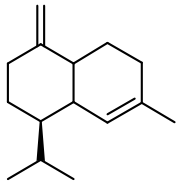
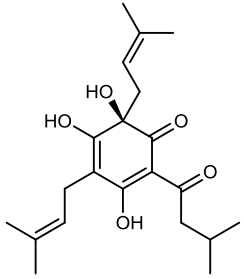
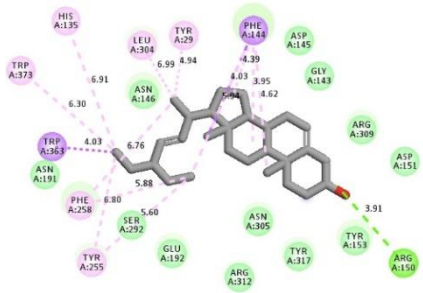
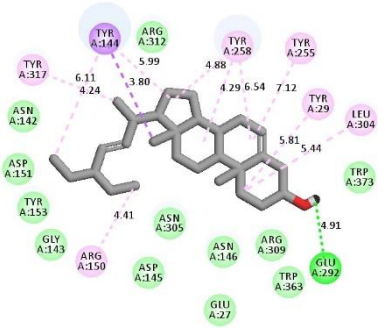
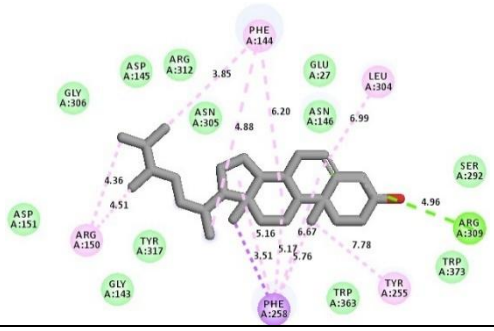
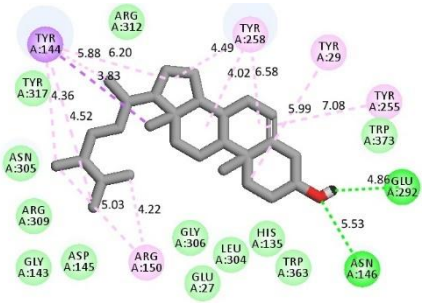
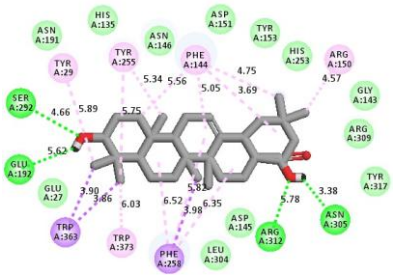
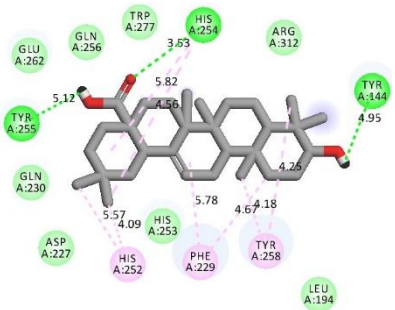
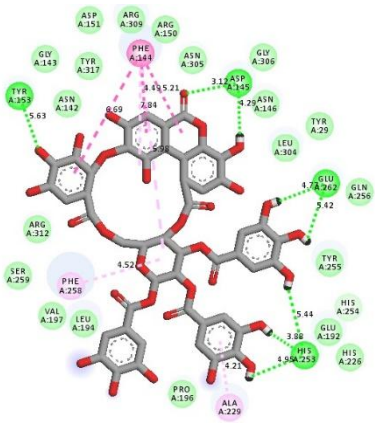
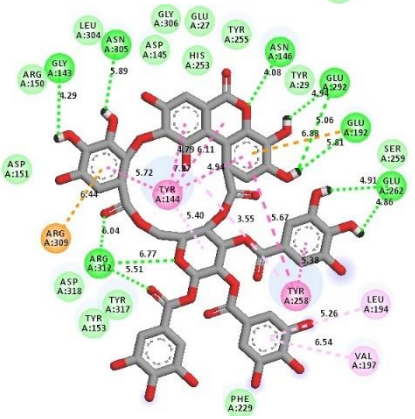
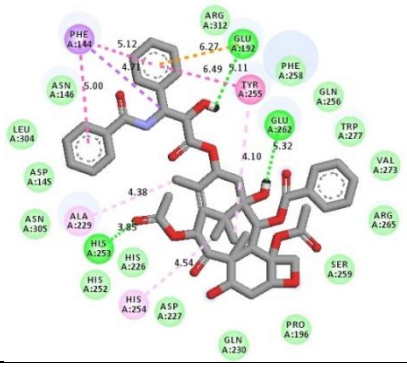
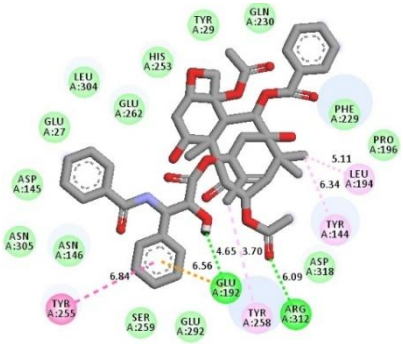
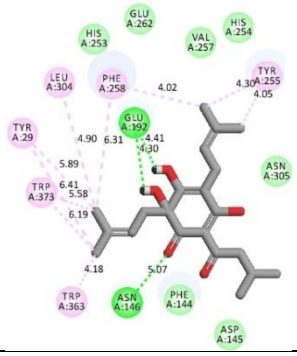
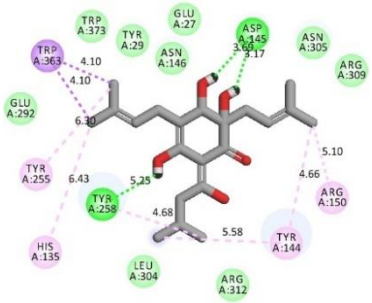
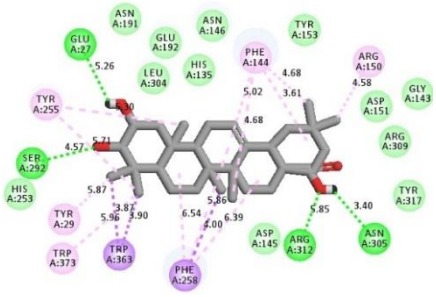
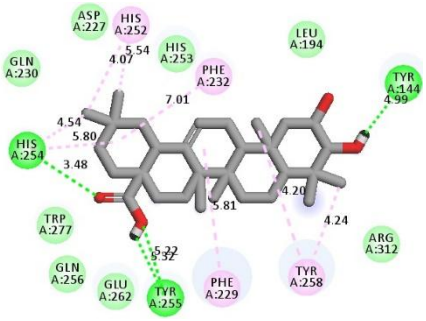
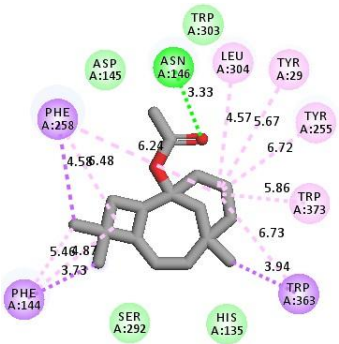
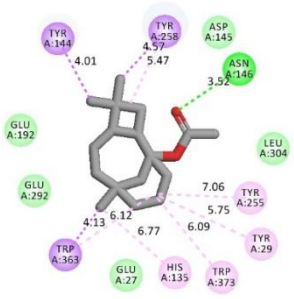
| | | | | | |
|---|---|---|---|---|---|
| Stigmasterol |  | -11.10 | 0.007 | -11.64 | 0.003 |
| Campesterol |  | -11.50 | 0.004 | -11.40 | 0.004 |
| Zingiberene |  | -7.45 | 3.48 | -6.67 | 12.98 |
| δ-Cadinene |  | -7.86 | 1.86 | -7.05 | 6.81 |
| Humulones |  | -9.31 | 0.149 | -8.78 | 0.366 |

**Table 2. 2D Binding mode of the best scores out of the 17 selected compounds, generated by discovery studio visualizer 16.1.**

| Compounds | *Candida Albicans* (3N9K) | *Candida Albicans* (3O6A) |
|---|---|---|
| Stigmasterol |  |  |
| Campesterol |  |  |
| Oleanolic acid |  |  |
| Bicornin |  |  |

| | | |
|---|---|---|
| Terpenoid(taxol) |  |  |
| Humulone |  |  |
| Maslinic acid |  |  |
| $B$-caryophyllene |  |  |

## 4. CONCLUSION

Considering the single ligand multiple sites bindings as an essentially procedure to define the binding interactions and differentiate between the selected compounds. The principle for all compounds is similar. The molecular docking results show that a similar binding interaction of stigmasterol and campesterol, and they are the best for *Candida albicans*' active site (3N9K and 3O6A). The hydroxyl moieties had played an important role in the antifungal potentiality of all selected compounds.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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