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In Silico Docking Studies of Bioactive Compounds in *Ocimum gratissimum* Essential Oil againstCandidapepsin-1 Enzyme from *Candida albicans*

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ABSTRACT

Antibiotic resistance in microorganisms has become a great challenge for pharmacists and research scientists. Crude extracts from plants used in traditional medicine could serve as an alternative source of resistance modifying agents due to the large number of different secondary metabolites contained in them. Literature survey has shown that the essential oil contained in *Ocimum gratissimum* has good antifungal activity against *Candida albicans*. However, little is still known about the bioactive compounds responsible for this activity. In this study, the essential oil in the leaf of *O. gratissimum* was extracted by hydrodistillation, and the compounds present in the oil identified using gas chromatography-flame ionization detection. Their inhibition potential against the fungal enzyme Candidapepsin-1 from *C. albicans* was studied using in silico methods. The docking results showed that the binding free energy of drimenin (–6.8 Kcal/mol), α-selinene (–6.6 Kcal/mol), and octamethylhexadecan-1-ol (–6.1 Kcal/mol) were close to the control drug Fluconazole (–7.4 Kcal/mol). Drimenin showed good ADME properties, making it a potential oral and dermal drug candidate to treat infections by this enzyme.

Keywords:*Ocimum gratissimum*, Hydrodistillation, Essential oil, Candidapepsin-1, Drimenin.

Introduction

The plant *Ocimum gratissimum* L. is a culinary herb of the Labiatae family, and is native to India and West Africa, where it is called African Basil.[1,2] It is found in the Savannah and coastal areas in Nigeria where it is called "Nchanwu" by the Igbos, "efinrin-nla" by the Yorubas, and "Dadoya" by the Hausas.[3,4] The presence of essential oil in the leaves of *O. gratissimum impacts the plant with a* unique fragrance.[5,6] Characterization of this essential oil has shown the presence of phytochemicals like eugenol, thymol, geraniol, *β*-caryophyllene, valencene, p-cymene, etc.[7] In traditional medicine, the crude extract from the leaf of this plant has been used to treat different ailments and diseases like high fever, cold, fungal infection, epilepsy, and diarrhea.[8] The essential oil has been reported to have pronounced antimicrobial activity against different bacteria and fungi.[9] It has also been incorporated in various bases as topical antiseptics in treating minor wounds, boils, and pimples.[10]

The Candidapepsin-1 is a harmful proteolytic enzyme from the endophytic polymorphic contagious species *Candida albicans*, that causes superficial Candida diseases like oral and skin infections in persons with compromised immune systems.[11,12] To obtain sustenance, the enzyme attacks, and locks onto the host tissue by digesting the host cell membrane and other small molecules. It also attacks the haemoglobin through its proteolytic action and releases different antimicrobial hemocidins to battle various microorganisms of the same niche.[13] The enzyme contains two chains (A and B) with an aggregate of 391 amino acid residues. The active site of the protein carries a net negative electrostatic charge because of basic amino acids. The active site, which is more extensive at the entry point, is situated between ASP82 and ASP267. Hence, molecular docking of bioactive compounds on this site can be studied for drug development. Information concerning the pharmacological applications of the essential oil from *O. gratissimum* leaves is ubiquitous in literature, with these studies focusing on the whole oil extract. However, there is little knowledge of the actual compounds responsible for the observed activities. In this research, the phytochemical composition of the essential oil from the leaves of *O. gratissimum was reported*. In silico approaches were used to investigate the activity of these compounds against the enzyme Candidapepsin-1 of *C. albicans.*

Materials and Methods

Identification of plant material and extraction of essential oil

Fresh *O. gratissimum* leaves were collected in August 2020 from a household garden in Owerri, Imo State, Nigeria. The identification of the leaf samples was done by a professional taxonomist Prof. F.N. Mbagwu of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The voucher specimen was deposited at the Imo State University Herbarium, with herbarium number IMSUH- 471. Thoroughly washed leaf samples (200 g) were sliced into small pieces and immersed in 500 mL distilled water in a 1 L flat bottom. The essential oil was extracted completely from the leaves by hydrodistillation, using a Clevenger system for 2 h at 100°C, and the collected essential oil was dried over anhydrous Na_2SO_4. The dried oil was separated from the drying agent by filtration and then stored in an amber essential oil vial at –20°C.[14]

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GC-FID analysis of essential oil

GC-FID analysis and quantification of phytochemical components in the essential oil were carried out using a Buck 530 gas chromatograph (GC) fitted with a flame ionization detector (FID). The instrument was equipped with an on-column, automatic injector, electron capture detector, and HP 88 capillary column (100 m × 0.25 µm film thickness). The oven temperature was programmed from 60 ^{o}C to 230^{o}C with injector temperature at 230 ^{o}C and detector temperature at 250^{o}C. The identification of compounds was based on the retention time, which is determined using the authentic standards. The integrated peak area is directly converted to concentrations in a user-defined unit, such as ppbv or µg/m^3. The calculations are performed by the data acquisition and processing station. All processed data were automatically compiled and reported through a data report function incorporated in the data station. The reported data includes the compound, name, retention time, and concentration.[15,16]

Identification and preparation of ligands

The 3D structure-data files (SDF) of the bioactive compounds in the essential oil were identified and downloaded from the PubChem database. They were minimized in PyRx virtual screening tool, using Universal Force Field at 200 steps. They were then converted to AutoDock ligands (pdbqt) and used for the docking analysis.

Identification and preparation of the molecular target

The Candidapepsin-1 enzyme (ID: 2QZW) with resolution 2.05 Å was identified from literature and used as a drug target in this study. The protein was retrieved from the Protein Data Bank (PDB) database and consisted of two chains, A and B. Chain A of the protein was selected for the docking studies to increase the ligand-binding precision.[17] The interfering crystallographic water molecules and minimization of the protein were done using UCSF Chimera 1.14.[18]CASTp (Computed Atlas for Surface Topography of Proteins)[19] was used to view the active sites and amino acid residues around the largest active site.

Docking and post-docking studies

Multiple docking of the ligands on a specified Candidapepsin-1 protein binding pocket was done with AutodockVina in PyRx software [20] (version 0.8). The center grid box sizes were *x* center: − 19.185, *y* center: −17.584, and *z* center: −20.888. The binding free energies of the compounds on the protein target were obtained after the docking process. Biovia Discovery studio 4.5 [21] was used to visualize the interactions between the protein-ligand complexes after the docking process.

Absorption, Distribution, Metabolism and Elimination (ADME) analysis

The most potent bioactive compound was chosen and sent to the SwissADME server to examine its drug-like properties[22] and compared them with those of the control drug.

Results and Discussion

The compounds identified in the GC-FID analysis and their concentrations are shown in Figure 2 and Table 1, respectively. Isobornyl acetate, octamethylhexadecan-1-ol, nonadecanal, decanal, undecanal, α-selinene, and drimenin with amounts 64.3 %, 12.2 %, 11.5 %, 8.7 %, 2.8 %, 0.5 %, and < 0.1 % respectively were the identified compounds in the oil.

The binding affinities of the compounds from the oil on Candidapepsin-1 protein were compared with that of Fluconazole the control drug and the values are shown in Table 2.

In the docking studies of components from the essential oil of *Trachyaspermum ammi* against Candidapepsin-1 enzyme, it was observed that the compound ligustillide had the highest binding affinity (–5.8 Kcal/mol) on the most extensive active site of this enzyme.[23] In this study, the binding affinity of drimenin (–6.8 Kcal/mol), α-selinene (–6.6 Kcal/mol), and octamethylhexadecan-1-ol (–6.1 Kcal/mol) were higher than what was reported for ligustillide on the same active site of Candidapepsin-1 enzyme. The binding position of drimenin and Fluconazole on the target enzyme is shown in Figure 3. The binding affinity of drimenin was very close to that of the control drug (–7.4 Kcal/mol), indicating that it could have similar activity as Fluconazole on the enzyme target.[24,25,26,27] Drimenin is a sesquiterpene commonly found in Canelo tree[28] (*Drimys winteri*). Isolates of this compound from *D. winteri* have been reported to have excellent antifungal activity against *Gaeumannomyces graminis* var. tritici.[29]This fungus that attacks the roots of grass and cereal plants in temperate climates. The interactions of drimenin and Fluconazole with the amino acid residues in the enzyme are shown in Figure 4.

Pi-Sigma interaction at TYR84 was the only force holding the drimenin molecule in the active site of the protein. A similar interaction was observed between Fluconazole and TYR84 but of a Pi-Pi stacked nature. The bond lengths of these interactions (3.94 Å and 4.81 Å for drimenin and Fluconazole, respectively) suggested that the Pi-Sigma was stronger than the Pi-Pi interaction. The presence of two hydrogen bonds between Fluconazole and ASP218, and interactions between this drug and other protein residues confirmed a higher binding affinity to the protein than drimenin.[30,31]

The pharmacokinetic and pharmacodynamic properties of drimenin and Fluconazole revealed by their ADME properties are summarized in Table 4. The drug likeliness of drimenin was assessed from Lipinski's Rule of Five and the Rule of three. Good drug candidates should not violate more than one of the rules.[32]The values were compared with those obtained for Fluconazole.

Drimenin has a molecular weight < 500, and its lypophilicity (Wlog P) was less than 3.7, which showed that the compound had good cell membrane penetration. The hydrogen bond donor (5 hydrogen) and hydrogen bond acceptor (not more than 10 hydrogen) of the compound agreed with the rule of five, while the rotatable bonds (not more than 3) in the compound were in line with the rule of three. These results indicated that drimenin is a potent inhibitor of the Candidapepsin-1 enzyme.

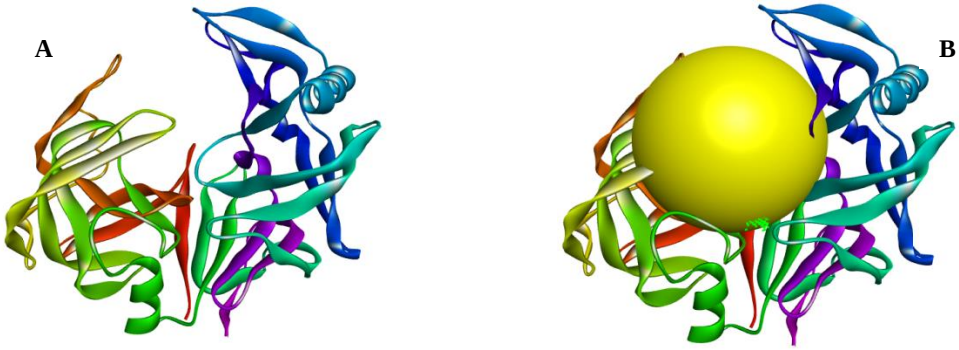

Figure 1: Candidapepsin-1 enzyme (A) Crystal structure (B) Position of the active site

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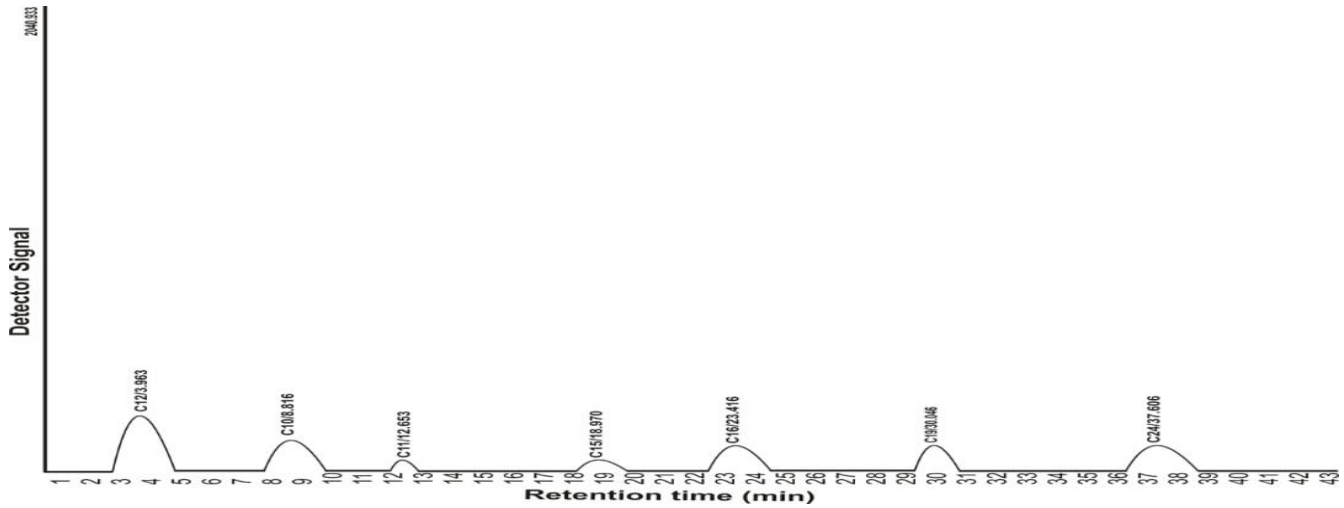
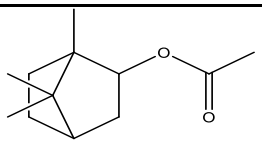
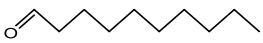
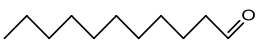
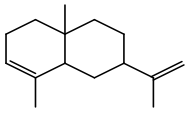
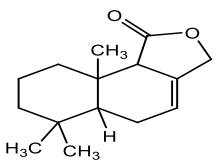


Figure 2: Gas chromatogram of compounds in *O. gratissimum* essential oil

Table 1: Identified compounds in the essential oil and their concentrations

S/N	Compound	Elution time	Concentration (µg/L)
1	Isobornyl acetate	3.96	510.31
2	Decanal	8.82	68.88
3	Undecanal	12.65	22.51
4	α-Selinene	18.97	4.17
5	Drimenin	23.42	0.13
6	Nonadecanal	30.05	91.30
7	Octamethylhexadecan-1-ol	37.61	96.76

Table 2: Binding free energies of the compounds on the protein target

Component	Structure	ΔG Energy (Kcal/mol)
Isobornyl acetate		-5.2
Decanal		-4.2
Undecanal		-4.4
α-Selinene		-6.6
Drimenin		-6.8

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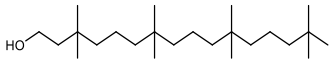
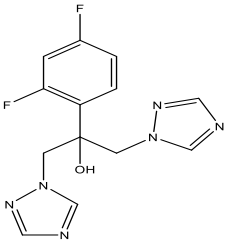
Nonadecanal		-5.0
Octamethylhexadecan-1-ol		-6.1
Fluconazole (control)		-7.4

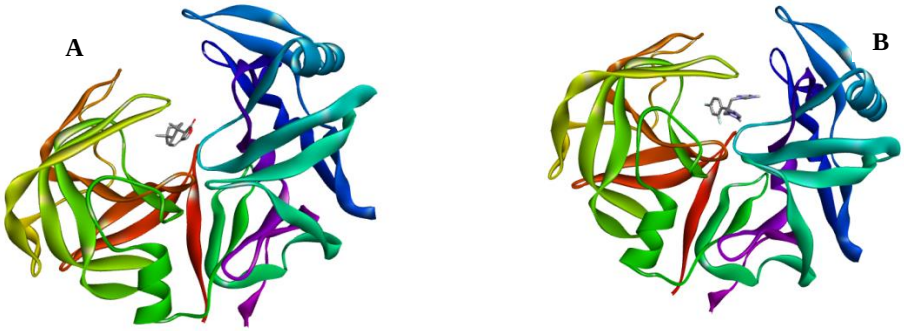


Figure 3: Binding positions of (A) drimenin (B) Fluconazole on the enzyme of Candidapepsin-1

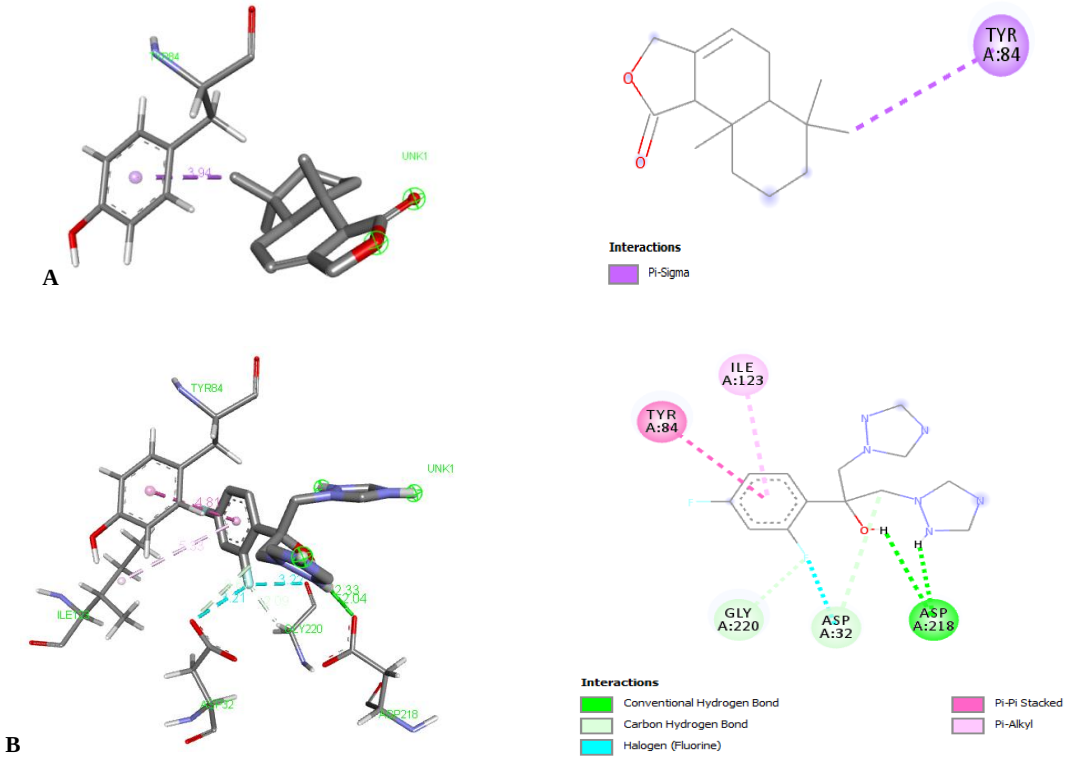


Figure 4: 3D (left) and 2D (right) views of **(A)** drimenin **(B)** Fluconazole interacting with protein residues

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Table 3: Protein residue interactions with drimenin and Fluconazole

Compound	Hydrogen bond	Carbon-Hydrogen bond	Pi-Sigma	Halogen	Pi-Pi stacked	Pi-Alkyl
Drimenin	-	-	TYR84	-	-	-
Fluconazole	ASP218	ASP32; GLY220	-	ASP32	TYR84	ILE123

Table 4: ADME properties of Drimenin and Fluconazole

Properties	Drimenin	Fluconazole
Molecular weight	234.33	306.27
WlogP	3.32	1.74
H-Bond Acceptor	2	7
H-Bond Donor	–	1
Rotatable Bonds	–	5

Conclusion

The composition and inhibitory properties of secondary metabolites in the essential oil from the leaves of *O. gratissimum* against candidapepsin-1 protein from *Candida albicans* were studied. Hydrodistillation was used to obtain the oil from the leaves of the plant, and the components were identified by GC-FID method. The essential oil extract contained seven different compounds. The in silico study of these compounds' activities against the enzyme Candidapepsin-1 showed that drimenin, followed by *α*-selinene and octamethylhexadecan-1-ol, respectively, had an excellent affinity for this protein. The ADME analysis of drimenin showed excellent pharmacokinetics and pharmacodynamic properties and therefore is a promising drug compound for the inhibition of Candidapepsin-1 enzyme from *C. albicans*.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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