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ORIGINAL ARTICLE

In silico drug-like properties prediction and *in vivo* antifungal potentials of *Citrullus lanatus* seed oil against *Candida albicans*

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KEYWORDS

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Abstract *Citrullus lanatus* seed is an important but neglected seed rich in essential fatty acids. The study sought to investigate *in silico* and *in vivo* antifungal activity of some bioactive compounds of *Citrullus lanatus* (watermelon) seed oil (CLSO) on oral candidiasis induced by *Candida albicans* in immunosuppressed female albino rats and to predict the Absorption, Distribution, Metabolism and Excretion (ADME) properties of isolated natural compound. Docking studies was performed using standard procedure, standard microbiological and histopathological techniques was employed for study of *in vivo* antifungal activity of the oil; as well as renal function tests at days 7 and 14 post-infection treatments. Treated groups were compared with that of the control groups. *In vitro* studies showed varied zone of inhibitions at different concentrations. Ligand-protein interaction showed better binding activity between Palmitic acid and SAP-5 as well as CYP51 when compared with fluconazole (reference drug). Treatment with CLSO showed that there was a significant reduction in the kidney fungal burden (cfu/ml/g) of rats treated with CLSO after 14 days post-infection treatment, compared to group 2 (untreated control) rats. Histomorphology of group 2 showed multifocal aggregation and widespread distribution of fungal blastospores when compared with CLSO-treated groups, which had minimal fungal blastospores in the renal tissues. Thus, *in silico* and histological data agreed with the findings in microbiology. Furthermore, within the CLSO treated group, a significant increase in the serum concentrations of creatinine was observed, while no sig-

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nificant difference in blood urea values was recorded after day 14 post-infection study. Linoleic and palmitic acid could be considered as a potential antifungal drug candidate with palmitic acid playing a significant role.

1. Introduction

Fungal infections such as candidiasis is becoming increasingly prevalent in the human population especially in immunocompromised conditions, and contribute to morbidity and mortality of the individuals (Drewniak et al., 2013). *C. albicans* under immunosuppressed condition causes opportunistic infections such as vaginal candidiasis and oral candidiasis. Most times, these fungi are capable of causing deleterious effect on the host by forming biofilms. Biofilms are formed through a biochemical pathway which entails seepage of Secreted Aspartic Proteinases (SAP) known for its virulence (Conti et al 2009). Exudation of Secreted Aspartic Proteinases (SAP) is one of the ways of determining different types of candidiasis. The SAP5 enzyme belonging to SAP family has been incriminated in fungal pathogenicity/virulence via a mechanism that permits colonization and infection of susceptible host. Therefore, an agent capable of obstructing the production of *C. albicans* SAPs will be a strong tool in management of *Candida* infections. One of the manifestations of impaired immune system is the expression of *Candida albicans* virulence in the oral cavity (Tamai et al., 2021). Studies have shown that *C. albicans* are resistant to azoles or polyenes, particularly amphotericin B (Arras and Usai, 2001; Giancarlo et al., 2021), which act by obstructing the lanosterol 14-demethylase (CYP51) enzyme, product of the (CYP51/ERG11) gene. Antimicrobial resistance has posed a serious threat towards treatment of fungal/fungal-related infection. In addition, Pierce and Lopez-Ribot 2013 reported that the current tools of antifungal agents is obviously not enough to meet the current challenges. In view of this, the discovery of new antimicrobial agents is of paramount importance. In order to avoid all these drawbacks, a search for new and effective alternative drugs is needed for the treatment of *C. albicans*-associated fungal infections. Molecular docking is one of the avenues used to understudy the ligand-proteins interactions that will lead to discovery of possible drug candidate. Understanding the process of drug discovery cannot be over emphasized in order to curb the challenges faced in the management candidiasis.

The antifungal effect of the seed oil of many plants has been described (Lee et al., 2021; Alves et al., 2020; Chami et al., 2004). *Citrullus lanatus* seed oil is rich in essential fatty acid (18:2*n*-6). The seed of watermelon which belongs to a class of cucurbit family and originated in Africa (Apeh et al. 2020). The seed is under-exploited and utilized in Nigeria despite its abundant medicinal values. The seed oil is rich in linoleic and palmitic acid (Apeh et al. 2020). Adewuyi et al. (2013) reported the antibacterial activity of biosurfactant of *Citrullus lanatus* seed oil. Similarly, Atolani et al (2020), had also reported the antioxidant and cytotoxicity of the seed oil. Keeping in mind the medicinal values of the oil, this study was undertaken to evaluate *in silico* and *in vivo* antifungal activity of *Citrullus lanatus* seed oil and predict the Absorption, Distribution, Metabolism and Excretion (ADME) properties of compounds.

2. Materials and methods

2.1. Plant materials

The plant material used in this study was the fresh *Citrullus lanatus* seeds. The seeds of *C. lanatus* (watermelon) was procured from the dealers within Zuba in Bwari area council of FCT Abuja, Nigeria. The identity of the plant was confirmed by Mr Felix Nwafor, while the Voucher specimens UNN/PSB/Consult/2019/2721-03 were deposited at the herbarium at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka,

2.2. Fungal and bacteria strains

Candida albicans strain (SC5314) strain used for this study was obtained from the Department of Microbiology University of Nigeria, Nsukka.

2.3. Collection and processing of Citrullus lanatus seeds

The fruits were washed, cut, and the seeds selected manually from the pulp. Tap water was used to wash off pulp on the seeds before sun-drying for 72 h. Dried samples of watermelon seeds were ground employing a commercial blender (TSK-949, WestPoint, France), preserved in an airtight container, and stored in a desiccator ready for analyses.

2.4. Oil extraction

The pulverized samples weighing 2480 g were extracted for 6 h with n-hexane in a soxhlet extractor equipped with a thimble (chem glass). Rotary evaporator (EYELA, N-N Series; Rikakikai Co Ltd., Tokyo, Japan) was used to concentrate the oil in order to obtain n-hexane-free *C. lanatus* seed oil. After concentration, crude *C. lanatus* was immediately placed in a hot air oven maintained at 50 °C for 24 h to ensure complete removal of solvents. The concentrated oil (642 ml) sample was then stored in air-tight bottle in a cool dry place until degumming.

2.5. Degumming of Citrullus lanatus seed oil

The oil was degummed according to the method of Zufarov et al (2008). The crude oil sample was heated to 80 °C, mixed with water (5% vol) and stirred for 15 mins by a magnetic stirrer. Then the mixture was centrifuged (7000 rpm) for 20 mins. Precautions were taken in order to prevent the introduction of

air and subsequent oxidation of the oil. The degummed oil supernatant was collected in sterile tubes after centrifuge.

2.6. Susceptibility assay (in-vitro)

Mueller-Hinton agar (Oxoid) plates supplemented with chloramphenicol were prepared for the antifungal activity assay, according to the CLSI agar well diffusion test (CLSI, 2019). Using sterile swab sticks, the standardized fungal cell suspensions (1×10^4 CFU/ml) were inoculated and spread onto the prepared agar surfaces. Within 15 mins of inoculation, wells of 6 mm in diameter were bored into the agar using sterile cork borer and the various oil concentration (63.5, 125.0, 250.0, and 500 mg/ml) were added into the wells using DMSO as a vehicle of choice. The plates were incubated at 37 °C for 24 h (bacteria) and 48 h (fungi).

Antibacterial activities of the oil extract were determined by the agar well diffusion method as described by Kim and Rhee (2016). Different concentrations of 63.5, 125.0, 250.0, and 500 mg/ml of the oil were used for the bioassay. Ciprofloxacin (5 µg) was used as standard antibacterial agent for positive control. After incubation, zones of inhibition formed in the medium were measured in millimeter (mm) diameter.

2.7. Result and interpretations

At the end of incubation, antimicrobial activity of both crude and degummed oil at a particular concentration was indicated by inhibition of growth around each well. The Zone of inhibition was measured with a meter rule in mm and recorded as inhibition zone diameter (IZD) of the oil.

2.7.1. Interpretation

The wider the IZD, the higher the antimicrobial activity of the oil, and the organism is reported as susceptible to the oil at the given concentration. While the absence of IZD implies no activity and the organism is reported to be resistant to the oil at the given concentration and antifungal activity of the crude and degummed *Citrullus lanatus* seed oil were determined according to the agar-well diffusion method described by Irobi et al. (1994).

2.8. Docking analysis

The software used includes OpenBabel Program 2.1.1 (downloaded from http://openbabel.org/), AutoDock Vina program (Molecular Graphics Laboratory, The Scripps Research Institute in 2011 downloaded from http://vina.scripps.edu/download. html), Structures of Secreted Aspartic Protein-5 (SAP5) (PDB ID: 2QZX) and lanosterol 14-demethylase (CYP51) (PDB ID: CYP51) enzyme was obtained from protein databank (www.rcsb.org). The protein targets retrieved from protein databank were uploaded to Chimera 1.14 workspace. All non-standard residues including ions, water molecules and co-crystallized ligands were removed. The protein structures were minimized at 200 steepest descent steps, 0.02 steepest descent steps size (Ă), 10 conjugate gradient steps, 0.02 conjugate gradient steps size (Ă), and 5 update intervals, using the structure editing wizard panel of Chimera 1.14. Solvents were removed, hydrogens were added, charges were assigned using AMBER ff14SB force field, histidine was set for the protonation state. All non-standard amino acids in the protein crystal structures were converted to standard amino acids. The output files were converted to PDBQT using software. Structure data file (SDF) structures of palmitic acid and linoleic acid and standard inhibitors were retrieved from the PubChem database.

OpenBabel plugin of Autodock tools was used to convert the proteins and ligand molecules to dockable pdbqt format. Docking of the ligands to various protein targets as well as determination of binding affinities was carried out using Autodock Vina plugin in PyRx software (Trott and Olson, 2010). BIOVIA Discovery studio 2020 and Chimera 1.14 were used to perform post docking analysis and visualization of the molecular interactions between the proteins and ligands respectively.

2.8.1. Admet screening

Absorption, distribution, metabolism, excretion and toxicity (ADMET) of the test compounds were determined using an *in silico* integrative model predicted at the SwissADME and PROTOX-II web servers respectively. Using a huge database, the servers speculate physicochemical properties, lipophilicity, water-solubility, pharmacokinetics, drug-likeness, medicinal properties and toxicity of compounds with high precision.

2.9. Animal material

A total of 72 female albino rats weighing 120–150 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. They were acclimatized for 7 days under standard environmental conditions and maintained on a regular feed and water ad libitum. They were grouped into 9 groups of 8 rats each and administered with crude and degummed *Citrullus lanatus* seed oil for a total of 14 days. Controls were treated with 2% Tween 80 and levamisole as standard drugs. The rats were sacrificed on the 14th day, blood samples used for biochemical analyses were collected through ocular puncture.

2.10. Candida albicans preparation

2.10.1. Preparation of Candida albicans for animal study

Stock cultures of *C. albicans* (SC5413) cells were kept at 4 °C and passaged regularly to maintain viability. Before each experiment, *C. albicans* (SC5413) cells were grown in Sabouraud's dextrose agar at 37 °C for 48 h. Cells were harvested by centrifugation at 3500*g* for 10 min. Cells were washed 3 times with 50 ml of sterile non-pyrogenic phosphate buffered-saline (PBS), counted with a haemacytometer, and resuspended in PBS to the required concentrations (1×10^7).

2.10.2. Induction of oropharyngeal infection with Candida albicans in rat

After 7 consecutive days of immunosuppression with 50 mg/kg bodyweight of pyrogallol, disseminated candidiasis infection was induced by oral (*p.o.*) administration of 0.2 ml of a 1×10^7 UFC/mL. Also, 1 g/ml of antibiotic (tetracycline) was administered *po* for 3 days (to eliminate competitive normal flora) during the inoculation of *Candida albicans*.

2.10.3. Animal grouping for virulence studies

A total of 72 female albino rats weighing 120–150 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. They were acclimatized for 7 days under standard environmental conditions and maintained on a regular feed (Grand Cereals Ltd, Enugu, Nigeria) and water *ad libitum*. The animals were handled according to the guidelines of the Ethical Committee on the use and care of experimental animals of the Department of Biochemistry, University of Nigeria, Nsukka (UNN) and approved by the Departmental Animal Ethics Committee (DAEC), UNN (Approval No. UNN/DAEC/2019/B79). They were grouped in nine (9) cages of eight (8) rats per cage and orally treated with crude and degummed *Citrullus lanatus* seed oil (CLSO) for a total of 14 days. Controls were treated with 2% Tween 80 while fluconazole and levamisole as standard drugs. The rats were sacrificed on the 14th day, blood samples were collected through ocular puncture for biochemical and hematological analyses.

2.10.4. Determination of Candida albicans in infected tissue homogenates

At 7 and 14 days of treatment, rats were euthanized using diethyl ether, and target organ (kidney) were excised and homogenized in 1 ml of sterile phosphate-buffered saline (PBS). Tissue homogenates from individual rats were serially diluted (10^{-1} and 10^{-2}) using PBS and cultured on SDA plates and incubated for 48 h at 37 °C prior to quantifying *C. albicans*. Results were expressed as CFU $\log_{10}$ per organ.

2.10.5. Calculation of colony forming unit/ml/organ

The initial weight of the sample (organ) was taken, then 1 ml of phosphate buffer saline (PBS) was used to homogenise the sample and serially diluted as stated above. Colonies that were counted on a plate/volume plated is multiplied by dilution factor (DF) and the values were then divided by gram tissue /ml original volume

$$\mathrm{CFU/g\ \ tissue} = \frac{\frac{\text{colonies}}{\text{ml}}\ \text{plated} \times \mathrm{DF}}{\text{g}\frac{\text{tissue}}{\text{ml}}\ \ \text{original\ \ homogenate}}$$

2.11. Histological examination

2.11.1. Tissue handling

The kidneys harvested from the rats were excised and immersed (fixed) in Bouin's solution for 24 h and transferred to neutral buffered formalin for routine processing. Fixed tissues were sectioned, embedded in paraffin, and stained with Perodic acid-Schiff stain (PAS).

2.11.2. Light microscopy

C. albicans infection was assessed by evidence of lesions and by hyphal/blastospore colonisation on the kidney (Swidergall and Filler, 2017) with a digital imaging system. The PAS stained slides were used to examine tissue injury by the quantification of the number and type/areas of necrotic lesions microscopically (hereinafter known as focal aggregation of fungal blastospores); and photomicrographs were taken using Motic™ 5 megapixels microscope camera at magnifications ×100, ×200 and ×400.

2.12. Renal function test

Renal function test was determined by the method of Kozitsina et al. (2014).

2.13. Statistical analysis

The results were analyzed using a statistical software package – SPSS Version 21. Data were expressed as mean ± standard deviation (mean ± SD). Where the variables to be compared are three or more, one-way analysis of variance (ANOVA) was used, the Duncan test was used for post-hoc. $p < 0.05$ was considered statistically significant

3. Results

3.1. *In vitro* antimicrobial test

Table 1 shows that crude and degummed oil (CO and DO respectively) of watermelon seed oil exhibited at different concentrations (63.5, 125.0, 250.0, and 500 mg/ml). There was inhibition against *Escherichia coli* at all the concentrations except for CO at 500 mg/ml while standard drug (Ciprofloxacin) showed inhibition of 14 mm higher than that of the test groups. However, there was only two inhibitions (6 and 8 mm) exhibited by CO at the concentrations of 250 and 500 mg/ml for *Staphylococcus aureus*, the inhibition by ciprofloxacin was at 8 mm. *Pseudomonas aeruginosa* resisted the antimicrobial activity of the oil at all concentrations while Ciprofloxacin inhibited *Pseudomonas aeruginosa* with a diameter of 10 mm. In the same vein, *Candida albicans* was sensitive to antifungal activity of CO at all concentration except at the concentration of 63.5 mg/ml; however *C. albicans* was sensitive to DO at all the concentrations of 63.5–500 mg/ml.

Docking analysis in Table 2 showed that fluconazole formed aromatic interactions with hydrophobic amino acid residues of secreted aspartic proteinases-5 (SAP-5). The residue interacting with linoleic acid formed strong π alkyl hydrophobic interactions with oxygen of the carbonyl group of the compound at a docking energy of −5.1 kcal/mol with amino acids of Tyr84, ILE30, ILE123, TRP51 and conventional hydrogen bond with ARG120. Residue ASP86 and linoleic acid formed unfavourable acceptor with the hydrogen of O—H group of linoleic acid. In another development, palmitic acid formed hydrogen bond interaction with hydrophilic ASP86 and Van der waals interaction with SER88 at the docking energy of −7.2 k/cal/mol. Fluconazole interacted with pi anion and aromatic ring that interacted with lone pair of pair of fluorine atom of residue ASP32 and GLY220 as well as pi-alkyl interaction at the binding pocket of ILE 123 at the binding energy of −6.8 kcal/mol (Figs. 1–3).

Table 3 show an interaction between ligands (linoleic acid, palmitic acid and fluconazole) and lanosterol 14-demethylase (CYP51) which indicated strong interactions leading to high binding affinities. Docking analysis showed a strong π alkyl hydrophobic interactions at the aromatic ring of PHE384, PHE241, LEU95, LEU96, TYR72, MET509 and PRO238 pockets that led to high binding affinity (−8.0 kcal/mol) with palmitic acid and formed another Van der waal interaction with the hydrogen bond of —OH group of palmitic acid. In

Table 1 Antimicrobial test using zone of inhibition as a marker.

Test organisms	Antibiotics	Inhibition zones diameter (mm)				
		500.0 mg/ml	250.0 mg/ml	125.0 mg/ml	63.5 mg/ml	5 µg/ml
E. coli	ECO	10 mm	6 mm	6 mm	5 mm	
ATCC 25922	EDO	11 mm	14 mm	12 mm	0 mm	
	Ciprofloxacin					14 mm
Staphylococcus aureus	ECO	0	12 mm	8 mm	0	0
ATCC 25923	EDO	12 mm	13 mm	3 mm	0	0
	Ciprofloxacin					8 mm
Pseudomonas aeruginosa	ECO	0	0	0	0	0
ATCC 15442	EDO	0	0	0	0	0
	Ciprofloxacin					10 mm
	ECO	13	10 mm	10 mm	0	0
Candida albicans	EDO	10 mm	16 mm	8 mm	10 mm	0
SC 5413	Fluconazole					11 mm

DO – Degummed oil, CO – Crude oil.

Table 2 The docking results of palmitic acid and linoleic acid to Secreted Aspartic Proteins-5 (2QZX).

Compounds	Protein Targets (secrete daspartic proteinases-5)	ΔG Energy (kcal/mol)	Amino acids
Linoleic acid	2QZX	−5.1	TRP51, TYR84, ILE30, ILE123, ARG120, ASP86
Palmitic acid	2QZX	−7.2	TYR84, ILE123, ASP86, SER88
Fluconazole (Inhibitor)	2QZX	−6.8	GLY220, ASP32, ASP32, ILE123

another development, the aromatic group of PHE384, PHE241 and HIS381 formed pi-pi- stacking interaction with fluconazole. The residues interacting with fluconazole formed pi-sigma interaction with aliphatic amino acid LEU95 and pi-alkyl hydrophobic interaction with amino acid PRO238 of two aromatic rings with a binding affinity of −6.2 kcal/mol. Furthermore, linoleic acid formed hydrophobic alkyl and pi-alkyl interactions only with amino acid PHE384, PHE241, LEU95, HIS381, TYR72, PRO238 of CYP51 at the binding energy of −6.8 kcal/mol (Fig. 6). Palmitic acid exhibited the best binding energy of −8.0 kcal/mol when compared with fluconazole (reference drug) which docked at the energy of −6.2 kcal/mol (Table 4, Figs. 4 and 5).

3.2. Effect of the crude and degummed oil on Candida albican colonization of kidney

The result of colony forming unit (CFU) {at 1 in 100 dilutions} counted in group 2 {suppressed, inoculated but not treated} was 689.67 ± 4.93 which shows significant increase ($p < 0.05$) when compared with groups 3 (326.67 ± 5.13), 4 (477.67 ± 3.51), 5 (278.33 ± 6.03), 6 (336.00 ± 4.00), 7 (273.33 ± 3.06) and 8 (186.00 ± 4.00). There was a significant increase ($p < 0.05$) CFU counted in group 4 when compared to group 3 (treated with 500 mg/kg b. w and 300 mg/kg b. w of degummed oil extract respectively). In the same vein, group 6 recorded a significant increase ($p < 0.05$) in CFU counted when compared with group 5 (treated with 500 mg/kg b. w and 300 mg/kg b. w of crude oil extract). It could be that the CFU recorded in group 9 (1.33 ± 0.58) was as a result

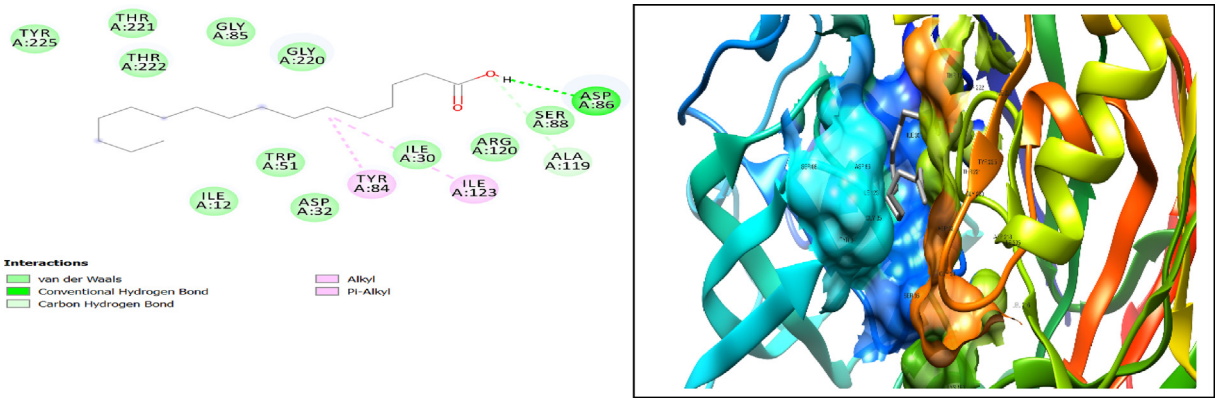

Fig. 1 2D and 3D representation of palmitic acid and 2QZX interaction involving Pi-alkyl and conventional hydrogen bond at amino acid residue of TYR84 and ILE123 as well as ASP86 respectively.

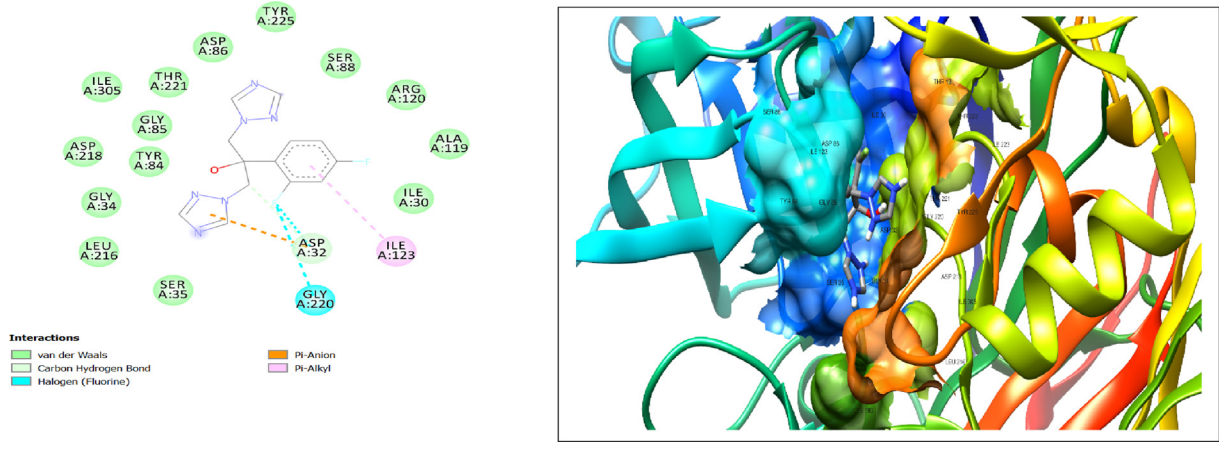

Fig. 2 2D and 3D representation of interaction between fluconazole and 2QZX involving amino acid residue of ILE123, ASP32 and GLY220.

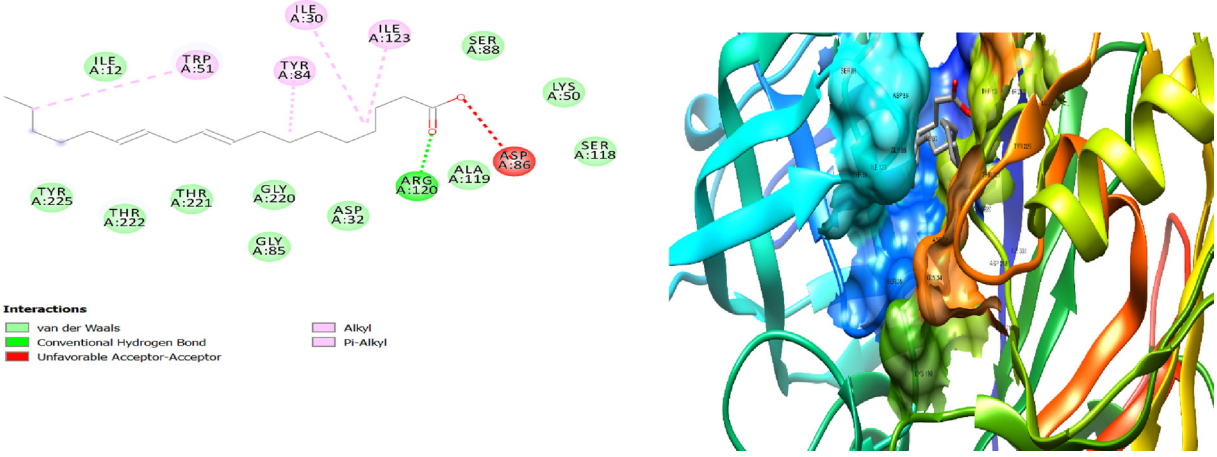

Fig. 3 2D and 3D representation of interaction between linoleic acid and 2QZX involving ILE30, ILE123, TYR64 and TRP51 as well as ARG120 and ASP86.

Table 3 The docking results of palmitic acid, linoleic acid and fluconazole to lanosterol 14_demethylase (CYP51).

Compounds	Protein Targets (lanosterol 14_-demethylase)	ΔG Energy (kcal/mol)	Amino acids
Linoleic acid	CYP51	−6.8	PHE384, PHE241, LEU95, HIS381, TYR72, PRO238
Palmitic acid	CYP51	−8.0	PHE384, PHE241, LEU95, LEU96, TYR72, MET509. PRO238, HIS381
Fluconazole (Inhibitor)	CYP51	−6.2	PHE384, PHE241, HIS381, LEU96, PRO238

Table 4 ADMET properties of selected compounds ADMET properties.

S/No	Property Name	Palmitic Acid	Linoleic Acid
1	Molecular weight (< 500 g/mol)	256.42 g/mol	280.4 g/mol
2	XLogP (< 5)	6.4	6.8
3	H- Bond Donor Count (< 5)	1	1
4	H- Bond Acceptor Count (< 10)	2	2
5	Topological Polar Surface Area (≤140 Å)	37.3 A^2	37.3 A^2
6	No of violations	1	1

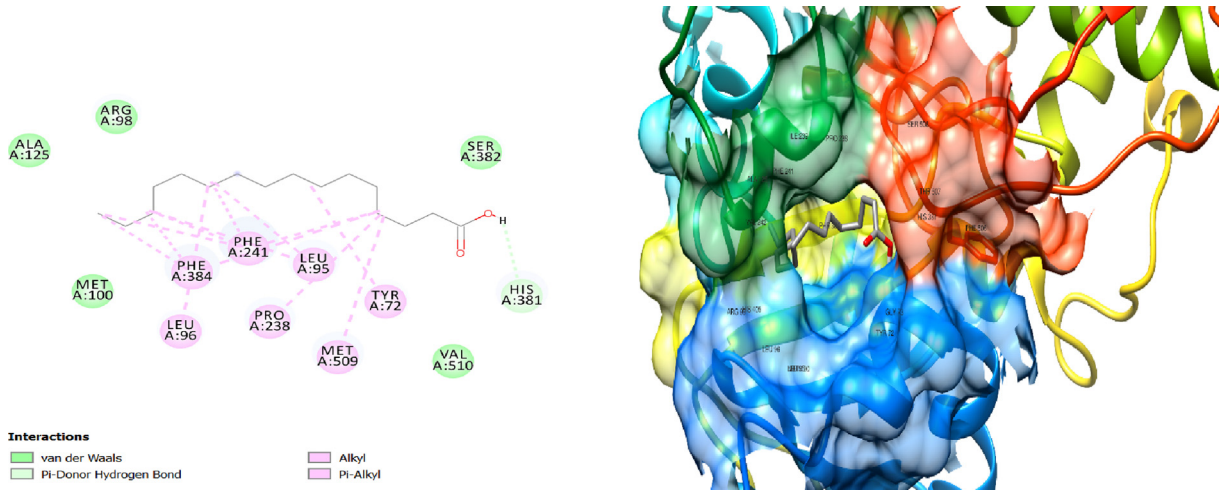

Fig. 4 2D and 3D structure of pi-alkyl hydrophobic interaction between palmitic acid and 5EAD involving amino acid residue of PHE384, PHE241, LEU95, LEU96, TYR72, MET509. PRO238.

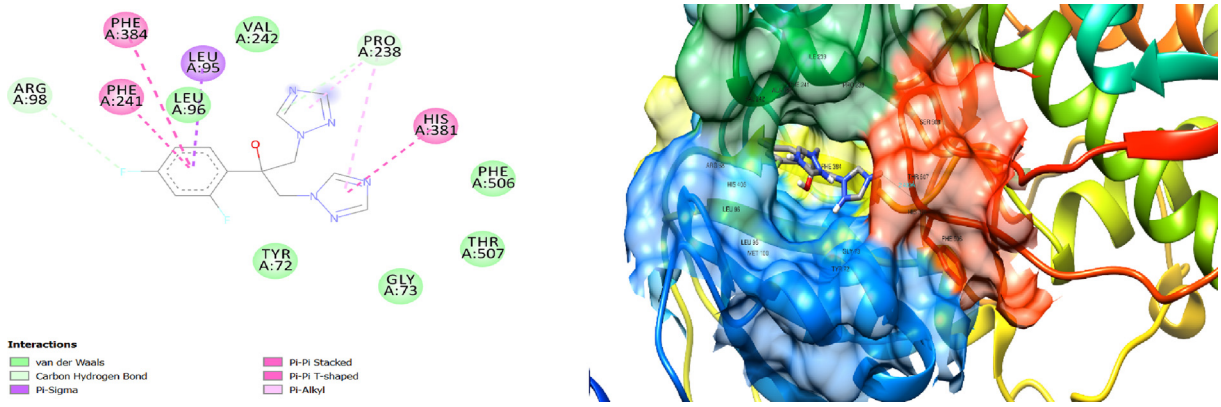

Fig. 5 2D and 3D structure of pi-sigma & pi-pi stacked interaction between fluconazole and 5EAD involving PHE384, PHE241, HIS381, LEU96, PRO238.

of contaminations, this is because it was only in one of the triplicates that gave four colonies. Groups 5 and 6 (crude) gave a lower ($p > 0.05$) count than groups 4 and 5 (degummed). When group 8 (treated with 5 mg/kg bw of Fluconazole) was compared with other treatment groups, it gave a marked decrease ($p < 0.05$) in CFU when compared with other treatment groups. It also revealed that increase ($p < 0.05$) in CFU recorded in group 2 was as a result of immunosuppression when compared with group 1 that was not suppressed.

At the 14th day, there was a significant increase ($p < 0.05$) {at 1 in100 dilutions} in CFU counted in group 2 (882.33 ± 8.50) when compared with groups 3 (145.33 ± 5.86), 4 (62.00 ± 18.52), 5 (165.67 ± 2.08), 6 (174.00 ± 2.65), 7 (226.33 ± 7.51) and 8 (210.33 ± 8.50). There was no significant difference ($p > 0.05$) between group 5 and 6 (treated with crude oil extract), same was the case between groups 7 and 8. While there was a reduction ($p < 0.05$) in CFU count in the groups treated with oil extracts, there was no significant reduction ($p > 0.05$) in CFU count in group 8 when compared with the 7th day CFU counts. When group 8 of day-14 was compared with group 8 of day-7, there was a slight increase ($p > 0.05$) in day-14 CFU count (Fig. 7).

Colony forming unit of 10^{-2} dilutions of tissue homogenate on SDA

Group 1 = inoculated without immunosuppression
Group 2 = inoculated + suppresses without treatment
Group 3 & 4 = inoculated + suppressed + treated with 300 & 500 mg/kg b.w.of DO respectively
Group 5 & 6 = inoculated + suppressed + treated with 300 & 500 mg/kg b.w.of CO respectively
Group 7 = inoculated + suppressed + treated with 25 mg/kg bw levamisol
Group 8 = inoculated + suppressed + treated with 5 mg/kg bw Fluconazole
Group 9 = Control

3.3. Result of body and kidney weight

After 7 days of treatment, there was a significant increase ($p < 0.05$) in the body weight of rats in groups 3, 4, 5 and 7 when compared with group 9 (normal control). This significant increase was sustained up to the 14th days of the study. There

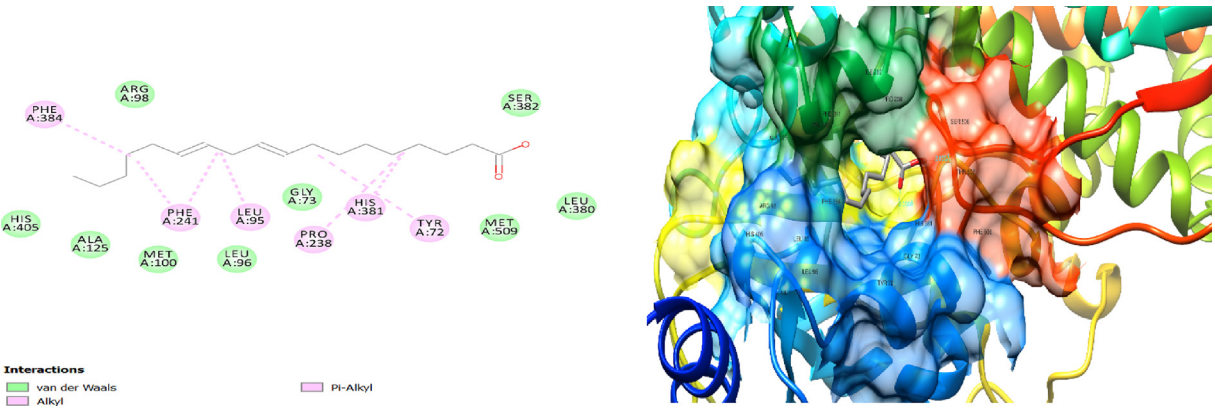

Fig. 6 2D and 3D structure alkyl and pi-alkyl interaction between linoleic acid and 5EAD involving amino acid residue of PHE384, PHE241, LEU95, HIS381, TYR72, PRO238.

were no uniform variations within the group and between the days of the study. The organ weight of all treated groups decreased between 7th and 14th day, except in groups 3 and 5 (which were treated with 300 mg/kg bw of degummed and crude oil extract respectively) which experienced a slight increase ($p > 0.05$). This could also imply that immunosuppression and *Candida albican* inoculation affected the kidney more than that of group 9 which was not suppressed at all thus were the only groups that experienced increase in organ weight after 14th day of the study (Table 5).

3.4. Effect of Candida albican on the renal function

There was no significant difference in urea value in all the treated groups when compared with the controls in the first 7 days. But the treated groups recorded a lower ($p > 0.05$) urea value when compared with group 9 {7.33 ± 2.58} (normal control). However, the trend slightly changed on the 14th day which recorded a significant increase ($p < 0.05$) in the treated groups as well as group 9 when compared with group 1 (not suppressed but inoculated). There was also an elevation of urea value in group 2 (immunosuppressed, inoculated but not treated) on day 14. But group 1 (24.47 ± 19.38) increased (though not significant) more than group 2 (18.27 ± 2.02) and group 2 increased more than the treated groups. Groups 7 and 8 that were treated with standard drugs Levamisol and Fluconazole respectively were also reduced (not significant) more than the treatment groups.

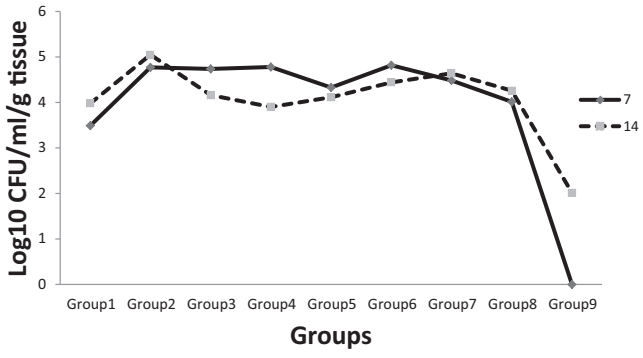

Fig. 7 Fungal Burden of Kidney Colonized with *C. albicans*.

After 7 days of treatment, the creatinine value gave a significant increase ($p < 0.05$) in all treated groups except group 4 (64.67 ± 16.33) when compared with groups 1 (42.43 ± 4.92) and 9 (48.91 ± 12.07). While on the 14th day, there was a significant increase ($p < 0.05$) in all treated groups when compared with group 1 (52.95 ± 12.06). But group 2 (117.73 ± 6.42) showed a significant increase ($p < 0.05$) when compared with the treated groups (Table 6).

3.5. Histopathologic findings

Histologic results revealed several features of the degenerative characteristics of *C. albicans*. Results demonstrated that the severity of tissue pathology is related to the integrity of the immune system and the concentration of *C. albicans* inoculated as observed in our pilot study.

3.5.1. Photomicrograph of C. albicans spores on the kidney in group 1 after 7 and 14 days

Plate 1(1): Sections of the kidney collected from the animals in this group showed the presence of blastospores that appeared within the renal parenchyma and glomeruli with minimal fungal activity. No degeneration and necrosis of the renal tubules with evident fungal blastospores were observed.

Plate 2(1): Sections of the kidney collected from the animals in this group showed no degeneration and necrosis of the renal tubules with no evident fungal blastospores. Likewise, the intra-renal blood vessels were devoid of fungal blastospores.

3.5.2. Photomicrograph of C. albicans spores on the kidney in group 2 after 7 and 14 days

Plate 1(2): Sections of the kidney collected from this group showed multifocal aggregation of fungal blastospores (arrow) appearing singly or in small clusters within the renal parenchyma. The distribution is widespread, involving the Glomeruli, lumen of renal tubules and blood vessels. In the renal tubules, spores are evident in the cytoplasm of degenerate epithelial lining cells. The blastospores are round to oval in shape, vary in sizes and occasionally show budding off of smaller spores.

Plate 2(2): Sections of the kidney collected from this animal showed multifocal aggregation of fungal blastospores (arrow) appearing singly as well as in clusters within the renal parenchyma. The distribution is random, involving primarily the renal

Table 5 Effect of *Candida albican* colonization and treatment on body and kidney weight.

Groups	Rat Weight day 7	Rat Weight day 14	Kidney weight day 7	Kidney weight day 14
Group1	132.25 ± 9.39^{abc}	142.65 ± 8.08^{ab}	0.61 ± 0.04 ^{cd}	0.79 ± 0.07^{e}
Group2	125.63 ± 7.80^{ab}	142.90 ± 13.61^{ab}	0.72 ± 0.06^{e}	0.54 ± 0.01^{b}
Group3	150.00 ± 9.46^{bc}	157.67 ± 14.65^{bc}	0.50 ± 0.01^{b}	0.61 ± 0.09^{bc}
Group4	151.34 ± 5.56^{bc}	158.13 ± 3.26^{bc}	0.69 ± 0.03^{c}	0.69 ± 0.04 ^{cd}
Group5	150.64 ± 17.58^{c}	159.18 ± 18.23^{bc}	0.57 ± 0.02^{c}	0.67 ± 0.05^{d}
Group6	129.97 ± 14.70^{abc}	135.89 ± 18.40^{ab}	0.66 ± 0.04 ^{cd}	0.59 ± 0.04^{bc}
Group7	141.17 ± 13.43^{bc}	150.80 ± 11.13^{bc}	0.67 ± 0.02 ^{cd}	0.68 ± 0.5 ^{cd}
Group8	134.83 ± 5.44^{abc}	151.01 ± 9.01^{bc}	0.71 ± 0.07^{e}	0.66 ± 0.03 ^{cd}
Group9	113.64 ± 10.17^{a}	125.52 ± 6.58^{a}	0.33 ± 0.02^{a}	0.38 ± 0.02^{a}

Body and organ weight of rats at day-7 and day-14:
Group 1 = inoculated without immunosuppression.
Group 2 = inoculated + suppresses without treatment.
Group 3 & 4 = inoculated + suppressed + treated with 300 & 500 mg/kg b.w.of DO respectively.
Group 5 & 6 = inoculated + suppressed + treated with 300 & 500 mg/kg b.w.of CO respectively.
Group 7 = inoculated + suppressed + treated with 25 mg/kg bw levamisol.
Group 8 = inoculated + suppressed + treated with 5 mg/kg bw Fluconazole.
Group 9 = Control.

tubules where the spores are evident in the cytoplasm of degenerate epithelial lining cells.

3.5.3. Photomicrograph of C. albicans spores on the kidney in group 3 at day-7 and 14

Plate 1(3): Sections of the kidney collected from this animal showed multifocal aggregation of fungal blastospores (arrow) appearing singly as well as in clusters within the renal parenchyma. The distribution is random, involving the renal tubules where the spores are evident in the cytoplasm of degenerate epithelial lining cells as well as in the renal pelvis.

Plate 2(3): Sections of the kidney collected from these animals showed mild fungal blastospores (arrow) mostly appearing singly within the renal parenchyma. The distribution is random, involving primarily the renal tubules where the spores are evident in the cytoplasm of degenerate epithelial lining cells. The blastospores are round to oval in shape, vary in sizes, and occasionally show budding off of smaller spores.

3.5.4. Photomicrograph of C. albicans spores on the kidney in group 4 at day 7 and 14

Plate 1(4): Sections of the kidney collected from this animal showed multifocal aggregation of fungal blastospores (arrow) appearing singly as well as in clusters within the renal parenchyma. The distribution is random, involving primarily the renal tubules where the spores are evident in the cytoplasm of degenerate epithelial lining cells. The blastospores are round to oval in shape, vary in sizes, and occasionally show budding off of smaller spores.

Plate 2(4): Sections of the kidney collected from this animal showed mild fungal blastospores (arrow) presence in the renal parenchyma. The few observed fungal blastospores mostly appeared singly within the Glomeruli and renal tubules.

3.5.5. Photomicrograph of C. albicans spores on the kidney in group 5 at day 7 and 14

Plate 1(5): Sections of the kidney collected from this animal showed minimal fungal blastospores (arrow) presence in the renal parenchyma. The few observed fungal blastospores appear singly within the Glomeruli and renal tubules. Glomeruli (G); Renal tubules (RT). PAS stain x400.

Plate 2(5): Sections of the kidney collected from this animal showed mild fungal blastospores (arrow) presence in the renal parenchyma. The few observed fungal blastospores mostly appeared singly within the Glomeruli and renal tubules.

Table 6 Effect of different treatments on Urea and Creatinine concentrations

Groups	Urea (mMol/L) day 7	Urea (mMol/L) day 14	Creatinine (μMol/L) day 7	Creatinine (μMol/L) day 14
Group1	9.52 ± 0.94^{b}	24.47 ± 19.38^{a}	42.43 ± 4.92^{a}	98.42 ± 8.22^{b}
Group2	6.55 ± .55^{a}	18.27 ± 2.02^{ab}	82.82 ± 3.34^{b}	117.73 ± 6.42^{c}
Group3	4.63 ± .83^{a}	10.11 ± 0.72^{b}	79.56 ± 4.02^{b}	99.23 ± 3.34^{b}
Group4	5.40 ± 1.20^{a}	10.37 ± 0.97^{b}	64.67 ± 16.33^{ab}	99.67 ± 7.23^{b}
Group5	5.13 ± 1.24^{a}	11.14 ± 3.33^{b}	84.86 ± 6.21^{b}	99.23 ± 6.55^{b}
Group6	5.47 ± 1.14^{a}	12.12 ± 3.30^{b}	85.76 ± 5.71^{b}	99.45 ± 6.55^{b}
Group7	7.50 ± 3.43^{ab}	9.13 ± 1.36^{b}	76.97 ± 8.63^{b}	100.48 ± 5.33^{b}
Group8	6.38 ± 1.79^{a}	9.21 ± 3.46^{b}	79.26 ± 2.84^{b}	100.48 ± 9.93^{b}
Group9	7.33 ± 2.58^{ab}	7.66 ± 0.70^{b}	48.91 ± 12.07^{a}	52.95 ± 12.06^{a}

3.5.6. Photomicrograph of C. albicans spores on the kidney in group 6 at day 7 and 14

Plate 1(6): Sections of the kidney collected from this animal showed multifocal aggregation of fungal blastospores (arrow) appearing singly as well as in clusters within the renal parenchyma. The distribution is random, involving primarily the renal tubules where the spores are evident in the cytoplasm of degenerate epithelial lining cells.

Plate 2(6): Sections of the kidney collected from this animal showed mild fungal blastospores (arrow) presence in the renal parenchyma. The few observed fungal blastospores mostly appeared singly within the Glomeruli and renal tubules.

3.5.7. Photomicrograph showing C. albicans spores dissemination on the kidney in group 7 at the 7th and 14th day

Plate 1(7): Sections of the kidney collected from this animal showed minimal fungal blastospores (arrow) presence in the renal parenchyma. The few observed fungal blastospores appear singly within the Glomeruli and renal tubules. Glomeruli (G); Renal tubules (RT). PAS stain ×400.

Slide 2(7): Sections of the kidney collected from this animal showed widespread multifocal aggregation of fungal blastospores (arrow) appearing singly as well as in clusters within the renal parenchyma. The distribution is random, involving the renal tubules where the spores are evident in the cytoplasm of degenerate epithelial lining cells. Glomeruli (G); Renal tubules (RT). PAS stain × 400.

3.5.8. Photomicrograph showing C. albicans spores dissemination the kidney in group 8 at 7th and 14th day

Plate 1(8): Sections of the kidney collected from this animal showed minimal fungal blastospores (arrow) presence in the renal parenchyma. The few observed fungal blastospores appear singly within the Glomeruli and renal tubules. Glomeruli (G); Renal tubules (RT). PAS stain x400.

Plate 2(8): Sections of the kidney collected from this animal showed widespread multifocal aggregation of fungal blastospores (arrow) appearing singly as well as in clusters within the renal parenchyma. The distribution is random, involving the renal tubules where the spores are evident in the cytoplasm of degenerate epithelial lining cells. Glomeruli (G); Renal tubules (RT). PAS stain ×400.

3.5.9. Photomicrograph showing C. Albicans spores on the kidney in group 9 (normal control)

Plate 9: Sections of the kidney collected from this animal showed the normal histomorphology of the kidney. No fungal activity was observed in both the cortex and the medulla as well as the pelvis.**

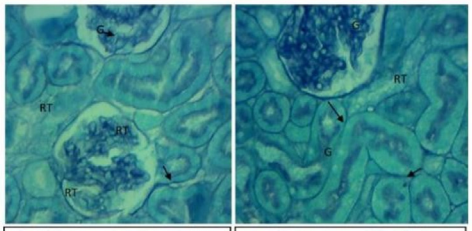

Plate1 (1): Cross section of the Kidney in Group 1 at 7th day of treatment

Plate 2(1): Cross section of the Kidney in Group 1 at 14th day of treatment

Glomerulus (G); Bowman's capsule (arrow); Renal tubules (RT). (PAS stain; × 400

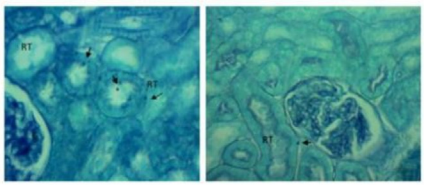

Plate 1(4): Cross section of the Kidney in Group 4 at 7th day of treatment

Plate 2(4): Cross-section of the Kidney in Group 4 at 14th day of treatment

Glomerulus (G); Bowman's capsule (arrow); Renal tubules (RT). (PAS stain; × 400).

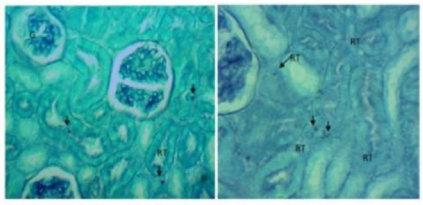

Plate 1(6): Cross section of the Kidney in Group 6 at 7th day of treatment

Plate 2(6): Cross section of the Kidney in Group 6 at 14th day of treatment

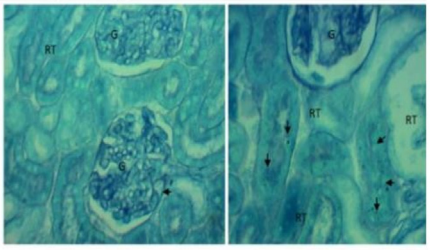

Plate 1(8): Cross section of the Kidney in Group 8 at 7th day of treatment

Plate 2(8): Cross section of the Kidney in Group 8 at 14th day of treatment

Glomerulus (G); Bowman's capsule (arrow); Renal tubules (RT). (PAS stain: × 400).

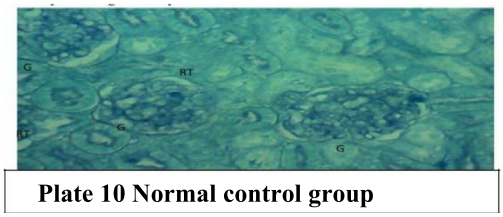

Plate 10 Normal control group

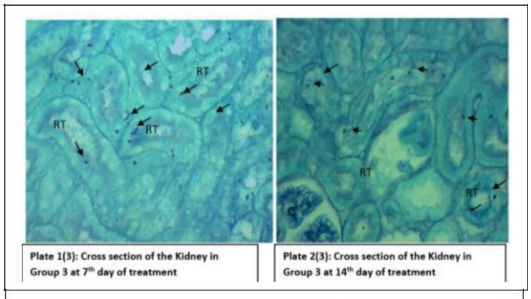

Plate 1(3): Cross section of the Kidney in Group 3 at 7th day of treatment

Plate 2(3): Cross section of the Kidney in Group 3 at 14th day of treatment

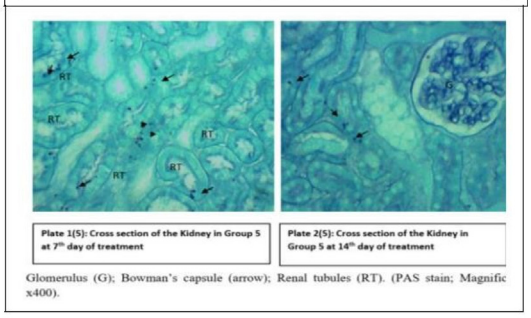

Plate 1(5): Cross section of the Kidney in Group 5 at 7th day of treatment

Plate 2(5): Cross section of the Kidney in Group 5 at 14th day of treatment

Glomerulus (G); Bowman's capsule (arrow); Renal tubules (RT). (PAS stain; Magnific x400).

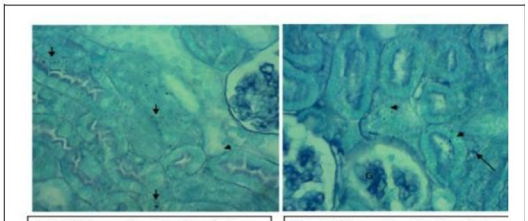

Plate 1(7): Cross section of the Kidney in Group 7 at 7th day of treatment

Plate 2(7): Cross section of the Kidney in Group at 14th day of treatment

Glomerulus (G); Bowman's capsule (arrow); Renal tubules (RT). (PAS stain: × 400).

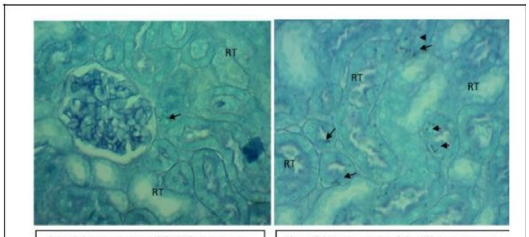

Plate 1(9): Cross section of the Kidney in Group 9 at 7th day of treatment

Plate 2(9): Cross section of the Kidney in Group 9 at 14th day of treatment

Glomerulus (G); Bowman's capsule (arrow); Renal tubules (RT). (PAS stain; Magnificati x400).

Glomerulus (G); Bowman's capsule (arrow); Renal tubules (RT). (PAS stain: ×400).

3.6. Discussions

The antimicrobial activity of both crude *Citrullus lanatus* seed oil (CO) and degummed *Citrullus lanatus* seed oil (DO) (Table 1) showed varied antimicrobial activity of the oils at different concentrations. The antimicrobial activity of both crude and degummed oil could be due to the activity of palmitic acid and linoleic acid in the oil as shown in Figs. 1–6, or maybe due to conventional hydrogen bond interaction of —OH group of the carboxyl group and ASP86 of Secreted Aspartic Protein 5 as shown in Fig. 1. It could also be due to inhibition of the enzyme activities in the microorganisms (Zheng et al., 2005). Free fatty acids are potent inhibitors of enzymes, unsaturated FFAs can also inhibit bacterial activity (Joffre et al., 2019). This could alter cell membrane fluidity and permeability causing destabilization of the membrane and leakage of the internal contents from the cell and death at the end. Zheng et al., (2005) observed the importance of —OH group in the antimicrobial activity of free fatty acid which is in line with docking analysis performed. Zheng et al. (2005) also reported that unsaturated FFAs has demonstrated greater antimicrobial action than saturated FFAs, however our docking analysis showed that palmitic acid exhibited the greatest binding affinity when compared with the reference drug (fluconazole). In another development Wille and Kydonieus (2003) reported that the position of double bonds can affect efficacy and spectrum of antimicrobial activity; the present docking studies also demonstrated that linoleic acid effectively inhibited lanosterol 14-demethylase (CYP51) enzyme which is an important component of *Candida albicans* membrane. Linoleic acid which has two double bonds were distinctly more active than other fatty acids with three double bonds (Giancarlo et al., 2021).

Body weight of an individual is influenced by the type and quality of food and other metabolic factors. There were no uniform variations in weight gain within the groups and between the days of the study. The organ weight of all treated groups decreased between 7th and 14th day, except in groups 3 and 5 (which were treated with 300 mg/kg b. w of degummed and crude oil extract respectively) which experienced a slight increase ($p > 0.05$) (Table 5). This could also imply that immunosuppression and *Candida albicans* inoculation affected the kidney more than groups1 and 9 which was neither suppressed nor treated. The reason for this development could not be understood but the oil might have reduced the rate at which food empties from stomach. Tanaka et al. (2008) indicated that administration (intra-gastric) of long-chain FFAs increased plasma cholecystokinin (CCK) levels, it was observed that intravenous injection of CCK into the vessels of the brain induces satiety (lack of hunger) in laboratory animals. Considering this mode of secretion relative to feeding, it would make a physiologic sense that this hormone plays a significant role in control of food intake in the rats treated with watermelon (*Citrullus lanatus*) seed oil which is rich in linoleic acid and palmitic acid. Sidhu et al., (2000) observed that fatty acid-induced CCK secretion in humans and from the enteroendocrine cell line which depends essentially on acyl chain length and that the potency of the fatty acid was directly proportional to its chain length; while both C10 and C12 stimulated CCK secretion over this range but C12 was significantly more effective. The chain length of linoleic acid and palmitic acid was above the minimum acyl chain length required to stimulate CCK secretion. CCK might have increased the sensation of short-term fullness, thereby reducing appetite during a meal. Invariably this suggests that the *Citrullus lanatus* seed oil could have appetite and weight reducing potentials.

Docking is of biological and pharmacological significance given its ability to predict the affinity and activity of the small drug molecules by allowing ligand position itself in a protein binding site. When linoleic acid and palmitic acid were docked against Secreted Aspartic protein-5 (SAP-5), the docking scores of the compounds as well as the standard drug were between −7.2 and −5.1 kcal/mol respectively. Palmitic acid achieved the highest binding affinity score of −7.2 kcal/mol, closely followed by fluconazole (standard drug) with a score of −6.8 kcal/mol. While linoleic acid interacted with SAP-5 at the energy of −5.1 kcal/mol. This suggests that palmitic acid is capable of inhibiting the production *Candida albicans* SAP-5 enzyme which is more virulent than other SAP strains. The interaction with hydrogen atom of —OH group could also be responsible for the high binding affinity of palmitic acid with SAP-5 enzyme. It has been suggested by Zheng et al (2005) that —OH groups could account for antimicrobial activity of free fatty acids.

While some amino acid residue enhances the activity of the ligands during ligand-receptor interaction, some downregulates the activity of ligands. Molecular docking study showed that the compounds and reference drug interacted with these same amino acid residues such as PHE384, PHE241, LEU96, PRO238 and HIS381. However palmitic acid exceptionally interacted with LEU95, TYR72 and MET509, this suggests that the antifungal action of palmitic acid is drawn from the ligand-receptor interaction at these amino acid binding pockets; giving it the best docking score. Similarly, linoleic acid exceptionally interacted with LEU95 and TYR72 to give it additional antifungal activity compared with the fluconazole. The best binding energies with lanosterol 14-demethylase (CYP51) were shown by palmitic acid and linoleic acid selected in this study (Table 3). The docking scores of palmitic acid and linoleic acid were −8.0 and −6.8 kcal/mol respectively while fluconazole was −5.2 kcal/mol.

Urea and creatinine measurements were used to evaluate renal function in rats challenged with pathogenic SC5314 strain of *C. albicans*. Increase in urea and creatinine in group 2 (when compared with its treated counterpart) could be due to immunosuppression and *C. albicans* infection. Marchenko et al. (2015) had reported that serum BUN level after *Candida* infection was significantly lower than that observed prior to the challenge. This report is in line with our findings. Renal disease is usually connected with reduced urea excretion and eventual increase in blood concentration. This result suggests that CLSO treatments could ameliorate the effect of *Candida albicans* on renal function when compared with group 3.

Creatinine showed a significant increase ($p < 0.05$) in all the treated groups when compared with group 9 on both the 7th and 14th day. There was no significant increase ($p > 0.05$) in creatinine concentration between 7th and 14th day. Both urea and creatinine are delivered from endogenous or exogenous protein but their independent rate of excretion denotes differing metabolic pathway. Our result also showed

that colonisation in the tissues and organs was minimised after 14 days of the study as shown in the histopathology studies.

In order to explore the invasive characteristics of *C. albicans*, we studied tissue microbiology and pathology in female albino *wistar* rats. This model is similar to human invasive infection occurring at gastrointestinal tract under immunocompromised condition; fungal cells are found in all organs, but the disease progresses mostly in the kidneys and brain (Ghosh et al., 2019). Kidney has been established to be one of the primary target organs in systemic candidiasis; it is also a major organ for the multiplication of *Candida* species (Ghosh et al., 2019). Therefore, the *in vivo* activity of *Citrullus lanatus* seed oil (CLSO) was investigated in experimental systemic candidiasis, by estimating the fungal burden in this organ histologically and microbiologically. The term "multifocal candidiasis" is used to describe the lesions/evasion in histopathologic diagnosis.

In group 1 during first seven (7) days, the number of viable *C. albicans* cells in the kidneys (homogenised and cultured on Saboraud Dextrose Agar) from animals that were inoculated but not treated nor immmunosuppressed, revealed a significantly low ($p < 0.05$) number *Candida albican* cells; after 14 days of treatment the number of viable count increased when compared with the group 2 (immunosuppressed and inoculated but not treated) which had a significantly higher ($p < 0.05$) number of viable *C. albicans* cells after 14 days period; suggesting that immunosuppression is a strong tool for multiplication of *C. albicans*. Similarly, histopathological slides showed a minimal fungal activity with no degeneration and necrosis of the renal tubules when compared to the group 2 which showed multifocal aggregation of fungal blastospores within the renal parenchyma which is also widespread in the glomeruli lumen of renal tubules and blood vessels with degenerate epithelial lining cells of the cytoplasm. It is believed that immunosuppression facilitated fungal colonization and evasion in group 2 rats. The suppression of immune status of the animals probably paved way for severe degeneration of epithelium and clusters of blastospores within the nephron; by enhancing fungal penetration of keratin epithelial linings and enhanced adherence (van de Veerdonk et al., 2010).

Groups 3 and 4 (immunosuppressed and treated with 300 and 500 mg/kg bw of degummed oil respectively) showed a significant decrease ($p < 0.05$) in colony forming units after 14 days of treatment when compared with group 2. This reduction in the blastospore could be due to antifungal activity of palmitic and linoleic acids as revealed in molecular docking (Tables 2 and 3). Linoleic acid and palmitic acid which are contained in oil has been reported to show antifungal activity (Annamaria et al., 2015). However, Group 5 and 6 (immunosuppressed and treated with 300 and 500 mg/kg b. w of crude oil respectively) showed a minimal fungal blastospore with single presence in the renal tubules and glomeruli; and also, a significantly reduced ($p < 0.05$) fungal burden in the colony counted when compared to group 2 after 14 days of treatment. Palmitic and linoleic acid restricted the penetration by hyphae and minimize inflammatory changes as well as inhibited colonization as shown in Figs. 1–6. The ameliorative effect witnessed in these treated groups could also be a result of the protective effect of antioxidant minerals (manganese, selenium, and zinc) present in both oils (Apeh et al. 2020). Pyrogallol may have also aided the oxidative stress and inflammatory reactions through its reducing properties leading to immunosuppression. Joharapurkar et al. (2004) had demonstrated that pyrogallol is an immunosuppressive agent through the induction of oxidative stress while Yu (2006) had observed that selenium and zinc inhibited oxidative stress and apoptosis.

In another development, it was observed that the animals showed some clinical presentations (swollen eyelid, persistent stridor, and occasional cough) during the experiment. This is purely a manifestation of respiratory tract infection suspected to have emanated from candidaemia. Stridor could be as a result of lesions coming from the central nervous system (CNS), the gastrointestinal (GI) tract, or the respiratory tract (April, 2016).

Group 8 (immunosuppressed and treated with fluconazole) showed minimal fungal blastospores increased at day-14 of post infection treatment. There could also be possible interaction between fluconazole and pyrogallol (reducing agent) which might mimic fluconazole at its receptor site(s). Findings suggest that fluconazole could act as a blockade to certain cytochrome P-450 isoenzymes involved in drug metabolism in humans (Debruyne, and Ryckelynck, 1993). Redding et al. (2003) reported that *Candida glabrata* does not respond to doses of fluconazole in cases of oral infection.

Group 9 (normal control: no *Candida albicans* inoculation and no treatment of any kind) showed the normal histomorphology of the kidney. No fungal activity was observed in both the cortex and the medulla. Likewise, no fungal activity was observed in the renal pelvis. In *C. albicans* cell counted, there was no count except for one single colony seen which might be suspected to be contamination.

This, therefore, has shown an increased level of colonization in immunosuppressed groups compared to *Candida* control (group 1); suggesting that increased risk of mucosal infections with *Candida albicans* was ameliorated by *Citrullus lanatus* seed oil as treatment progressed. Systemic/physiologic factors antagonistic to *Candida* organisms could constitute a barrier that inhibited fungal penetration apart from treatment with the oil; such factors might be absent in immunosuppressed rats and this allowed the fungus to superficially colonize the organs.

4. Conclusions

Citrullus lanatus seed oil has shown potent antimicrobial properties through the activity of isolated compounds. Having fallen within the Lipinski's rule of five, linoleic and palmitic acid could be considered as a potential antifungal drug candidate with palmitic acid playing a significant role. They can be considered to be orally available

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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