Applied Biological Research 25(3): 291-302; (2023)
DOI: 10.5958/0974-4517.2023.00034.4

IN SILICO STUDIES ON THE BINDING AFFINITY OF ROSMARINIC ACID, ALPHA TOCOPHEROL AND THEIR DERIVATIVES WITH CYP51 OF *Candida* SPECIES AND THEIR DRUG LIKELINESS POTENCIES

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(Received 13 March, 2023; accepted 25 June, 2023)

ABSTRACT

Various drugs are available to combat *Candida* infections by targeting sterol 14α-demethylase (CYP51) enzyme involved in ergosterol biosynthesis. However, most drugs have limitations due to the adversities and development of drug resistance by *Candida* species. Our study aimed at exploring natural alternatives in combating *Candida*-based infections for which antifungal efficacy of some selected antioxidants was evaluated using computational biology tools. The herbal molecules rosmarinic acid (RA), methyl rosmarinic acid (MRA), α-tocopherol (AT) and α-tocopherol acetate (ATA) were used as ligands for docking with CYP51 of *C. albicans* and *C. glabrata* using PatchDock docking tool. The drug likeliness analysis was done using Lipinski filter and AdmetSAR. The binding efficiency of RA, MRA, AT, ATA and fluconazole for CYP51 of *C. albicans* were observed to be -302.25, -346.74, -393.33, -442.80 and -280.21, respectively, while for CYP51 of *C. glabrata* it was -309.89, -324.47, -428.24, -464.5 and -256.6, respectively. All the selected molecules showed better binding affinity to CYP51 in both *Candida* species with respect to fluconazole, and also satisfied the criteria of drug likeliness. The present study demonstrated the potent drug likeliness of the four molecules that may contribute in designing novel *in vivo* therapeutic strategies against *Candida* infections.

Keywords: Antioxidants, binding affinity, *Candida*, CYP51, docking

INTRODUCTION

Candida albicans is a prime causative organism of candidiasis and causes severe infections (Kaleli *et al*., 2007). The *C. albicans* is typically a commensal residing in the buccal cavity and alimentary tract of humans. Apart from *C. albicans*, the non-albicans *Candida* species like *C. glabrata, C. krusei*, *C. tropicalis, C. parapsilosis,* etc. also cause infections at an alarming rate (Hoyer, 2001; Krcmery and Barnes, 2002; Tournu, 2005). *Candida* species are even considered as a chief cause of hospital-acquired infections (Berman and Sudbery, 2002; Maschmeyer, 2006).

Most antifungal drugs existing in market target ergosterol biosynthetic pathway. Of the several enzymes involved in ergosterol biosynthesis, sterol 14α-demethylase (CYP51), an intermediary enzyme, is considered a key antifungal drug target; and azoles available in market target CYP51 (Scorzoni *et al.,* 2017). However, these antifungal agents have side effects like hepatotoxicity and nephrotoxicity, besides human pathogenic fungi have developed resistance (Gupta and Tomas, 2003; Cannon e*t al.*, 2009). Hence, it is essential to search safer alternatives which may broaden the spectrum of activity against *Candida* species and combat resistant strains to conventional antifungal agents.

Several natural products are promising alternative agents for the treatment of candidiasis (Agarwal *et al.,* 2010; Shokri *et al.,* 2011). Of these, rosmarinic acid (RA) and α-tocopherol (AT) are

potential antimicrobial agents (Campoccia *et al.*, 2015; Hickl *et al.*, 2018). RA, an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, is considered a polyphenol and is usually found various plants of family Lamiaceae like *Rosmarinus officinalis, Coleus blumei, Prunella vulgaris, Perilla frutescens* and *Mentha aravensis* (Lu and Foo, 1999; Jayanthy and Subramanian, 2013). Rosmarinic acid has anti-allergic, antioxidant, anti-cancerous, anti-inflammatory, antibacterial and antifungal activities (Hickl *et al.*, 2018). Methyl rosmarinate (MRA), a methyl ester of rosmarinic acid, is one of the simplest derivatives of RA and possesses antioxidant, antihepatitis, antibacterial and antifungal properties (Suriyarak *et al*., 2013; Shi *et al*., 2016; Suriyarak *et al.*, 2018;). α-tocopherol (AT) is one of the most studied biologically active isoforms of vitamin E and comprises of a chromanol ring and an isoprenoid chain (Serafini, 2000). Vitamin E, especially AT, is found in numerous plant and animal products namely oily seeds, vegetable oils, wheat germ, dark-red vegetables, liver, and egg yolk.

In animal studies vitamin E has shown no mutagenic, teratogenic, and carcinogenic properties (Serafini, 2000) and possesses antibacterial and antifungal properties (Cheah and Gan, 2001). α-tocopheryl acetate (ATA), an acetic ester of AT, is one of the simplest derivatives of AT which is a thick, sticky, hydrophobic oil with an enhanced stability in comparison to the unreduced form (Beijersbergen *et al.*, 1995). α-tocopheryl acetate is commonly used to treat atopic dermatitis and is shown to have antimicrobial activities (Campoccia *et al.*, 2015). The RA, AT and their derivatives reportedly exhibit anti-candidal activities (Krzeminska *et al.*, 2002; Belhachemi *et al.*, 2021) but their effects on ergosterol biosynthesis have not been elucidated. Seitz (1979) has reported ergosterol as a measure of fungal growth and an indicator of fungal biomass production. Hence, the present study was aimed to evaluate the effect of RA, AT and their derivatives against the key regulatory enzyme CYP51 of ergosterol biosynthesis in *C. albicans* and *C. glabrata* using computational tools. The binding affinity of these test ligands were determined based on geometric shape complementarity score, approximate interface (AI) area and atomic contact energy (ACE) in the docking analysis.

MATERIALS AND METHODS

Retrieval of CYP51 enzyme structure

The crystal structures of cytochrome P450 14α-sterol demethylase (CYP51) [from *Candida albicans*], in complex with heme porphyrin ring (containing Fe) and itraconazole, with ID 5v5z (DOI: *10.2210/pdb5V5Z/pdb*) and from *Candida glabrata*, in complex with heme porphyrin ring (containing Fe), penta-ethylene glycol, triethylene glycol, chloride ion and itraconazole, with ID 5jlc (DOI: *10.2210/pdb5JLC/pdb*) were retrieved from Protein Data Bank (*http://www.rcsb.org/pdb/home/ home.do*). The details of the structure of the enzymes under the study were obtained using the PDBSum server.

Retrieval of ligand structures

The structures of test ligands RA (*https://pubchem.ncbi.nlm.nih.gov/compound/5281792*), MRA (*https://pubchem.ncbi.nlm.nih.gov/compound/6479915*), AT (*https://pubchem.ncbi.nlm.nih.gov/compound/14985)*, ATA *(https://pubchem.ncbi.nlm.nih.gov/compound/2117*), and FA (*https://pubchem.ncbi.nlm.nih.gov/compound/3365*) were retrieved from PubChem database and used as ligands for docking analysis. The 3D structures of selected ligands were downloaded from PubChem database in Structure Data file (SDF) format and converted to Protein Data Bank [PDB] format using online server (*https://cactus.nci.nih.gov/translate/*). Fluconazole, a known antifungal drug, was used as standard reference in docking analysis.

Optimization of the enzyme and ligands

The selected ligand and crystal structures of CYP51 enzyme were optimized by using the dockprep tool in UCF Chimera, molecular visualization tool, prior to docking. (Pettersen *et al*., 2004). Using UCF Chimera the ligands in the PDB structures of CYP51 of *C. albicans* (ID: 5v5z) and *C. glabrata*

(ID: 5jlc) were eliminated before the energy optimization. The optimized CYP51 structures and ligands were then used for docking analysis.

Prediction of prominent binding sites in CYP51

Metapocket 2.0 server was used for the prediction of significant binding sites in CYP51 before docking analysis (Huang, 2009; Zhang *et al*., 2011). The top three binding pockets in CYP51 structures were recovered by using Metapocket 2.0 server to identify the residues in the active site and their interaction with the test ligands.

Drug likeliness prediction of ligands

The drug likeliness of selected ligands viz., RA and its derivative MRA, and AT and its derivative ATA was predicted by using Lipinski filter (*http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp*). As per Lipinski filter a compound is said to act as a drug if it complies with at least two of the said criteria namely molecular mass <500 D, high lipophilicity (LogP < 5), less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors and molar refractivity between 40-130 (Lipinski, 2004; Jayaram *et al*., 2012). Additionally, some important properties of ligands *viz*., adsorption, distribution, metabolism, excretion and toxicity (ADMET) were analysed by using AdmetSAR (*http://lmmd.ecust.edu.cn/admetsar2/*) (Cheng *et al*., 2012; Yang *et al*., 2019).

Docking analysis

PatchDock server, a molecular docking algorithm based on shape complementarity principles, was used for docking of selected ligands with CYP51 (Schneidman-Duhovny *et al*., 2005). The PDB structures of CYP51 and ligands were uploaded on PatchDock server and docking analysis done by clustering RMSD of 1.5 and protein-small ligand complex as analysis parameters. PatchDock yields 1000 docked complexes with their geometric shape complementarity (GSC) score, approximate interface (AI) area and atomic contact energy (ACE). The docked solutions were sorted on the basis of GSC score. Of the top 100 complexes the solution with least ACE was selected for further analysis. ACE indicates the binding affinity of ligand with protein. Lower the ACE more is the binding affinity of a ligand with protein. The UCF Chimera program was used for interactive visualization of CYP51 and ligand docked complexes.

RESULTS AND DISCUSSION

Two biologically potent natural compounds with antioxidant properties *viz*., rosmarinic acid and alpha tocopherol were evaluated for their binding affinity with CYP51 enzyme. The enzyme CYP51 was chosen as target since it is a key enzyme in biosynthetic pathway of ergosterol (Fig. 1) which is a bio-marker for fungal growth and biomass (Seitz**,** 1979; Kosuri *et al*., 2018). Earlier rosmarinic acid was reported to show inhibitory effect on the growth of *C. albicans* ATCC 10231 and *C. glabrata* ATCC 2001 (Krzemińska *et al*., 2002; Kernou *et al*., 2023). Alpha-tocopherol reportedly enhances the therapeutic index of azole drug against *Candida*

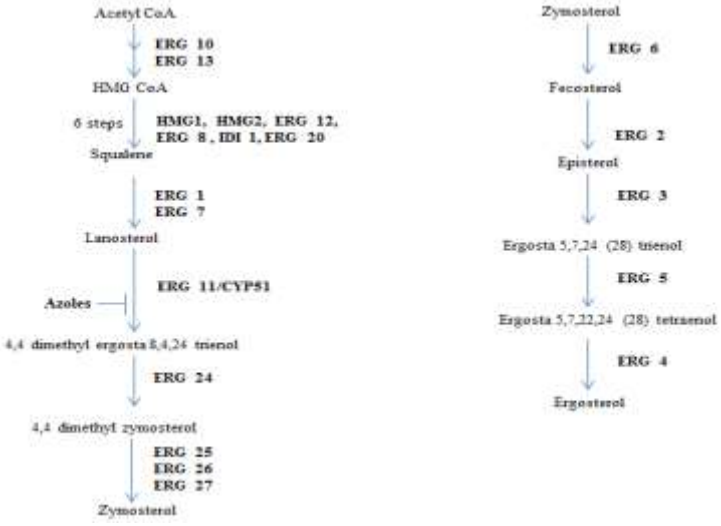

Fig. 1: Schematic representation of ergosterol biosynthesis

species (Belhachemi *et al.*, 2021). Although these studies revealed the anti-candidal potency of RA and AT, their role in influencing the ergosterol synthesis in the *Candida* species has been analyzed in our present studies computationally.

Analysis of CYP51 enzyme structure

The crystal structures of CYP51 of *C. albicans* and *C. glabrata* were obtained from PDB (Fig. 2). The details of secondary structure containing sheets, β-bulge and hairpin, strands, helices, helix interactions β and γ turns, relating to CYP 51 enzymes acquired from PDBSum server are given in Table 1. The data retrieved from PDBSum was further applied in analysing the structural assembly of protein and the flexible sites in CYP51 that are inclined for docking with ligands (Laskowski *et al.*, 1997).

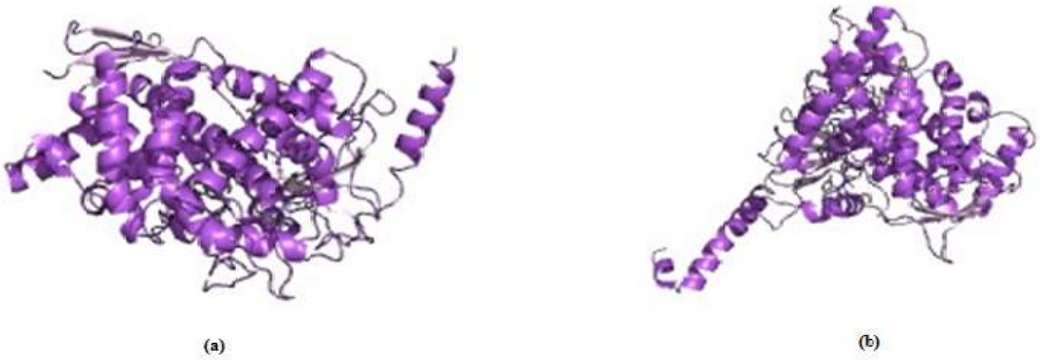

Fig. 2: Crystal structures of Cyp51 of (a) *C. albicans* (b) *C. glabrata*

Table 1: Structural details of target enzymes

Proteins	Sheets	β-hairpins	β-bulges	Strands	Helices	Helix-helix interactions	β-turns	γ-turns
CYP51 *(C. albicans)*	5	4	1	15	23	40	38	4
CYP51 *(C. glabrata)*	5	4	1	14	22	39	36	3

Drug likeliness prediction

The structures of RA, MRA, AT and ATA were retrieved from PubChem database (Fig. 3). Lipinski filter and AdmetSAR are widely used tools in predicting drug likeness (Cheng *et al.*, 2012). The test

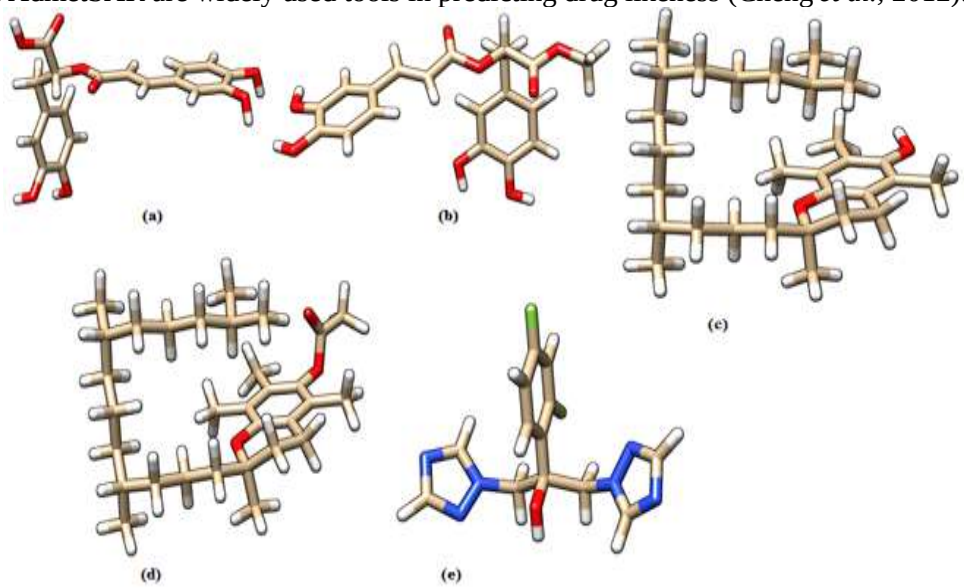

Fig. 3: 3D structures of the ligands retrieved from PubChem database: a) Rosmarinic acid, b) Methyl rosmarinic acid, c) α-tocopherol, d) α-tocopheryl acetate & e) Fluconazole

Table 2: Drug likeliness of the selected antioxidants

	RA	MRA	AT	ATA	FA
Lipinski filter					
Mass (dalton)	360.000	374.000	430.000	472.000	306.000
No. of H-bond donors	5	4	1	0	1
No. of H-bond acceptors	8	8	2	3	5
Log P	2.862690	1.849700	8.840264	9.059964	0.735800
Molar refractivity (m^3 mol^{-1})	86.848473	94.177170	134.390778	144.035049	70.29879
Criteria satisfied	All 5	All 5	3, except Log P & molar refractivity	3, except Log P & molar refractivity	All 5
ADMET analysis					
Ames mutagenesis	-ve	-ve	-ve	-ve	-ve
Acute oral toxicity	III	III	III	IV	III
Blood brain barrier	-ve	-ve	+ve	+ve	+ve
Biodegradation	-ve	-ve	-ve	-ve	-ve
Caco-2 permeability	-ve	-ve	+ve	+ve	+ve
Human intestinal absorption	+ve	+ve	+ve	+ve	+ve
Human oral bioavailability	-ve	-ve	+ve	-ve	+ve
P-glycoprotein substrate	-ve	-ve	-ve	-ve	-ve
Carcinogenicity	-ve	-ve	-ve	-ve	-ve
CYP inhibitory promiscuity	-ve	-ve	-ve	-ve	-ve

+ ve: indicates positive score; -ve: indicates negative test score

ligands RA and MRA satisfied all the five criteria while AT and ATA satisfied three criteria of drug likeliness (Table 2). As per Lipinski filter a compound is said to act as a drug if it complies with at least two of the said criteria namely molecular mass <500 D, high lipophilicity (LogP < 5), less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors and molar refractivity between 40-130 (Lipinski, 2004; Jayaram *et al.*, 2012). In present study all test ligands proved to have potent drug likeliness and thus contribute in designing novel therapeutic strategies for further study (*in vivo*) against *Candida* infections. Additionally, the properties such as adsorption, distribution, metabolism, excretion and toxicity of these ligands were analysed using AdmetSAR (Table 2). The results revealed that none of the ligands were suitable substrates for P-glycoprotein (P-gp). P-glycoprotein is an ATP dependent transporter that extrudes its substrates from the cytosol to the outside of cells in a uni-direction manner and thus limits the uptake of broad range of structurally varied compounds (Prachayasittikul and Prachayasittikul, 2016). With regard to the metabolism, we observed that the selected ligands were non-inhibitors of CYP450. A non-inhibitor of CYP450 points out that the molecule would not hinder the CYP450 catalysed biotransformation of drugs (Nisha *et al.*, 2016). The ligands displayed negative Ames mutagenesis indicating that they are non-mutagenic. Similarly, a negative carcinogenicity and biodegradation was displayed by the ligands. In terms of toxicity RA, its derivatives and AT were found to be slightly toxic whereas ATA was nontoxic. In comparison to FA all the five ligands for the parameters mentioned in the respective prediction tools, showed values suggesting the potency of their utilization as drugs in biological systems.

Docking analysis of RA, AT and their derivatives

The potential binding sites in CYP51 of *C. albicans* and *C. glabrata* were identified using Metapocket 2.0 server which revealed three binding sites (site 1, site 2 and site 3) in CYP 51 of *C. albicans* and *C. glabrata*. Further, to illustrate the binding efficiency of RA, AT and their derivatives with CYP51, the ligands were studied for docking using PatchDock server. Docking of selected ligands with target enzymes (CYP 51) was examined for certain parameters *viz*., GSC score, AI area, and ACE (Table 3 and 4). The ACE values of the complexes obtained on docking of CYP51 with RA, MRA, AT and ATA were lower and AI area was greater in comparison to the CYP51-FA docked complex, indicating

Table 3: Docking analysis of rosmarinic acid and its derivatives with CYP51 of *C. albicans* and *C. glabrata*

Ligand	GSC score	AI area	ACE Kcal mol^{-1}	Interacting residues
				Candida albicans
RA	3712	540.70	-302.25	Thr 122, His 377, Phe 233, Met 508, Tyr 118, Phe 380, Pro 230, Leu 121,Tyr 132, Phe 228, Ser 507, Tyr 64
MRA	4552	592.40	-346.74	Gly 303, Gly 307, Ile 304, Met 508, Phe 233, Phe 380, Tyr 118, Tyr 132, Leu 376, Thr 311
FA	3862	494.50	-280.21	Gly 307, Ile 131, Leu 376, Phe 228, Thr 122, Tyr 132, Leu 121, Gly 303, Phe 126, Thr 311, Hem Ho1
				Candida glabrata
RA	4000	520.40	-309.89	Val 513, Tyr 127, Thr 510, Pro 380, Phe 385, Phe 242, Met 512, Leu 381, Leu 130, His 382, Gly 74, Tyr 73, Ser 511, Ser 383, Pro 239, Phe 509
MRA	4344	558.80	-324.47	Gly 311, Ile 140, Leu 381, Met 512, Phe 135, Phe 237,Phe 242, Tyr 141, Gly 315, Ser 511, Thr 319,Tyr 127, Hem
FA	3966	496.20	-253.60	Gly 311, Gly 315, Leu 381, Met 314, Phe 135, Phe 237, Thr 131, Thr 319, Ile 140

Table 4: Docking analysis of alpha tocopherol and its derivative with CYP51 of *C. albicans* and *C. glabrata*

Ligand	GSC score	AI Area	ACE Kcal mol^{-1}	Interacting residues
				C. albicans
AT	4758	759.00	-393.33	Gly 307, Ile 131, Leu 121, Leu 376, Met 508, Phe 126, Phe 228, Phe 233, Phe 380, Thr 122, Thr 311, Tyr 118, Tyr 132, Val 509
ATA	5064	829.20	-442.80	Gly 307, His 310, Ile 304, Leu 121, Leu 376, Met 508, Phe 126, Phe 228, Phe 233, Thr 122, Thr 311, Tyr 118, Tyr 132, Val 509, Gly 303
FA	3862	494.50	-280.21	Gly 307, Ile 131, Leu 376, Phe 228, Thr 122, Tyr 132, Leu 121, Gly 303, Phe 126, Thr 311, Hem Ho1
				C. glabrata
AT	4678	751.20	-428.24	Leu 130, Leu 381, Met 512, Phe 237, Phe 385, Pro 239, Ser 383, Ser 511, Thr 319, Tyr 127, Val 513, Tyr 141, Leu 384, Thr 131
ATA	5070	810.10	-464.53	Leu 130, Leu 381, Met 512, Phe 237, Phe 385, Pro 239, Ser 511, Thr 319, Tyr 127, Val 513, Leu 384, Thr 131, Gly 311, His 318, Ile 140, Phe 135, Phe 242, Thr 238, Gly 315
FA	3966	496.20	-253.60	Gly 311, Gly 315, Leu 381, Met 314, Phe 135, Phe 237, Thr 131, Thr 319, Ile 140

that the RA, AT and their derivatives have more binding affinity and greater area of contact to the target enzyme than FA (Fig. 4b, d). The ACE score reflects the change in desolvation energy of a protein from unbound form to the complex state. A lesser ACE value implies a lower (more favourable) desolvation free energy (Guo *et al.*, 2012). Further, the GSC scores of MRA, AT and ATA docked complexes were more as compared to the FA docked complex; whereas the score of docked complex containing RA as ligand was comparable to that of CYP51 docked with FA individually (Fig. 4a, c). These outcomes point out that RA, AT and their derivatives bind more effectively to the CYP51 than the reference standard drug FA. Moreover, the effect of selected ligands on CYP51 of both *C. albicans* and *C. glabrata* was similar indicating that there is no species variation in their binding efficacy (Fig. 5a, b). The amino acid residues of CYP51 of *C. albicans* and *C. glabrata* with which RA, AT and their derivatives interact were identified by using UCF Chimera (Fig. 6 and 7). As discussed earlier the residues that interact with test ligands were found to be distributed in three binding sites of CYP51

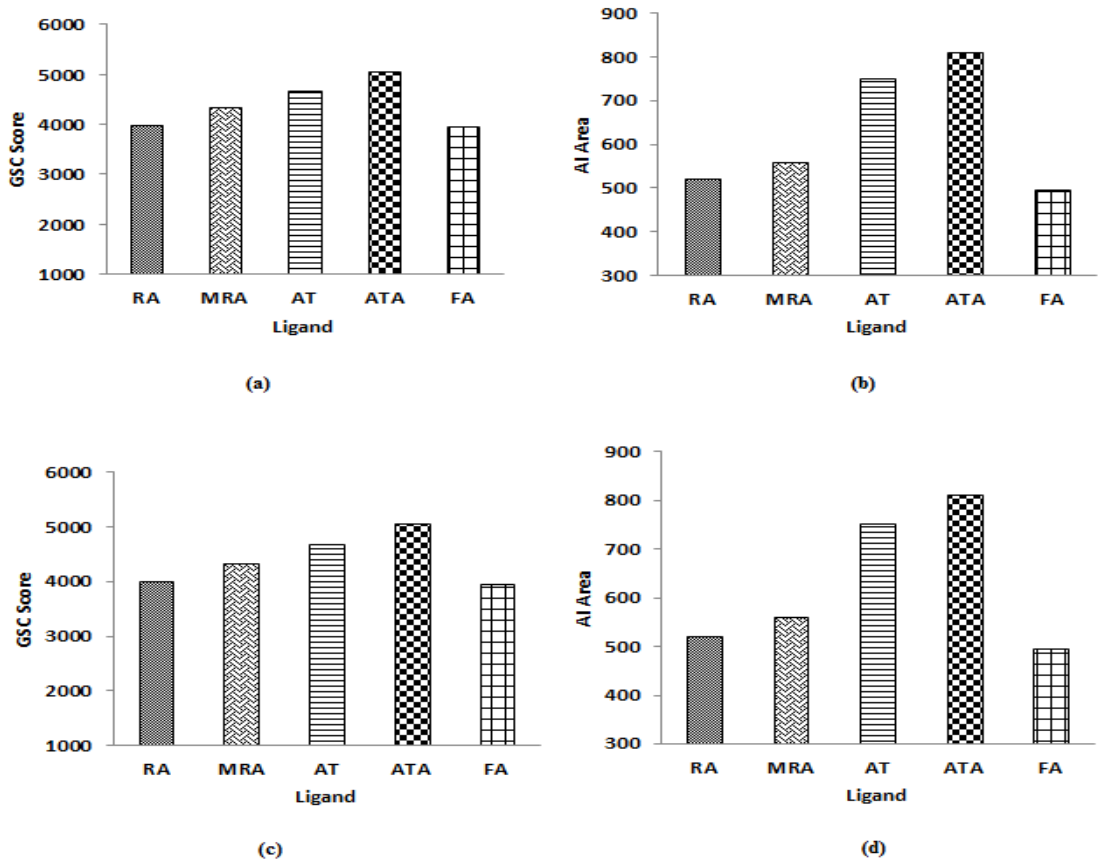

Fig. 4: Docking evaluation of RA, AT and their derivatives using Patchdock on the following parameters. a) GSC score & b) AI area of ligands docked with CYP51 of *C. albicans*, c) GSC score & d) AI area of ligands docked with CYP51 of *C. glabrata*

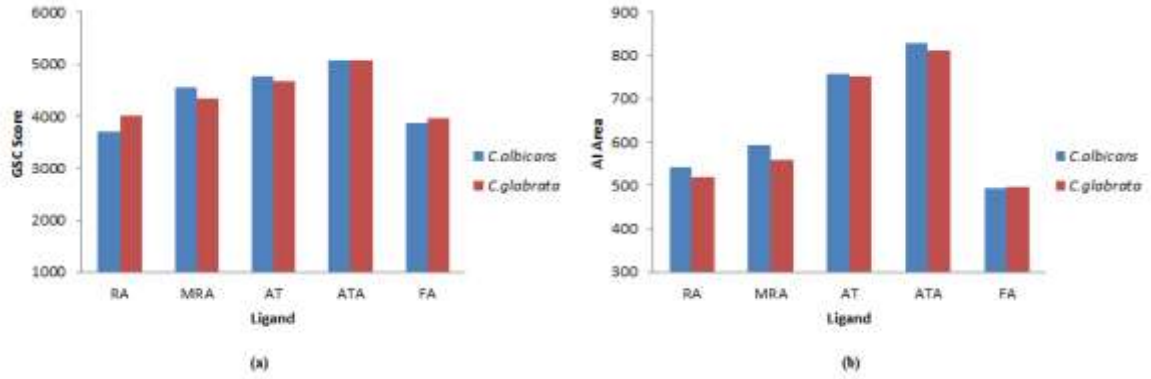

Fig. 5: Comparison of docking parameters (a) GSC score (b) AI Area of ligands docked with CYP51 of *C. albicans* & *C. glabrata*

of *C. albicans*. Rosmarinic acid was found to interact with residues in two binding sites, site 1 and site 2 whereas MRA, AT and ATA were found to interact with the residues in all the three binding sites CYP51 of *C. glabrata* (Table 5 & 6). The docking analysis revealed that Gly, Leu, Thr, Met, Tyr and Phe are the most frequently observed amino acids at the predicted docking sites in case of CYP51 of *C. albicans* and Gly, Leu, Ser, Thr, Met, Tyr and Phe in case of CYP51 of *C. glabrata*. The presence of these amino acids at the docking positions implies their importance in defining their binding strength. The above-mentioned amino acids are mostly involved in the hydrophobic interactions as no H-bond formation has been observed except for RA docked CYP51 complexes of *C. glabrata.*

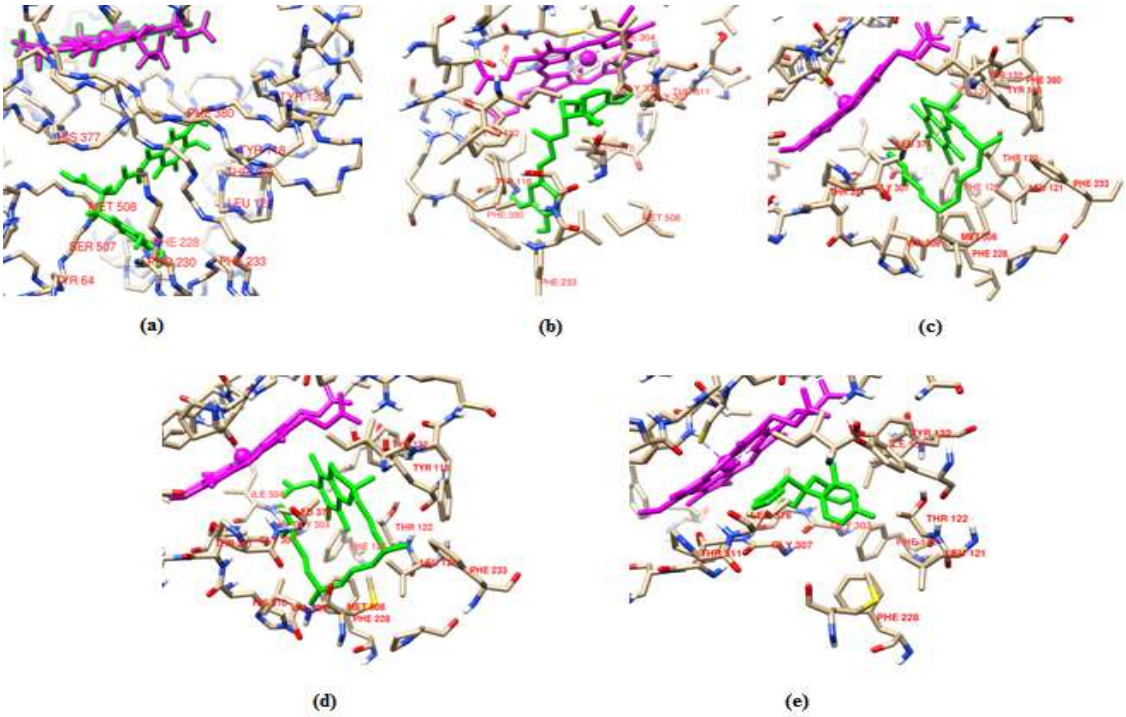

Fig. 6: Visualization of docked complexes of ligands with CYP51 of *C. albicans.* HEM porphyrin with iron: Magenta colour; Interacting amino acid residues: Red colour; Ligands: Green colour a) Rosmarinic acid, b) Methyl rosmarinic acid, c) α-tocopherol d) α-tocopheryl acetate, & e) Fluconazole

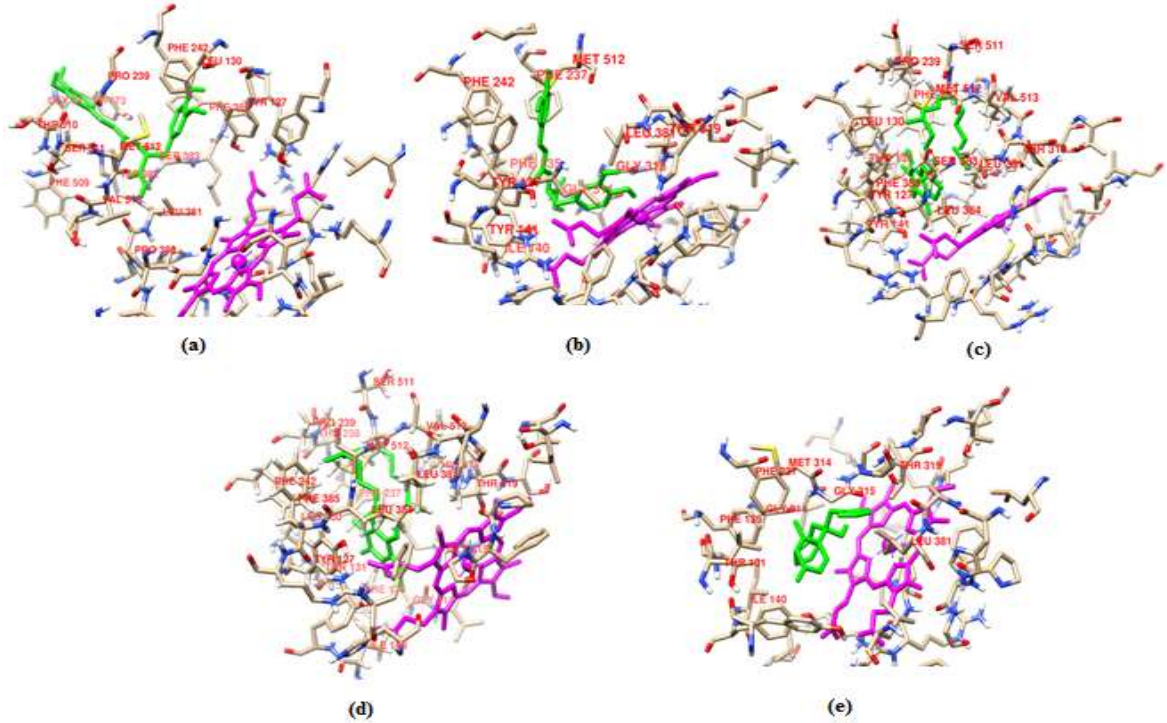

Fig. 7: Visualization of docked complexes of ligands with CYP51 of *C. glabrata;* HEM porphyrin with iron: Magenta colour; Interacting amino acid residues: Red colour; Ligands: Green colour; a) Rosmarinic acid, b) Methyl rosmarinic acid, c) α-tocopherol, & d) α-tocopheryl acetate; & e) Fluconazole

Table 5: Docking analysis depicting residues in CYP51 of *Candida albicans*, interacting with the ligands and presence of the residues in the reported binding sites

Ligand	Interacting residues of CYP51 (*C. albicans*)	Presence of interacting residues in binding sites in CYP51 (*C. albicans*) predicted by Metapocket	Ligand & protein atoms involved in H bonding
RA	Thr 122, His 377, Phe 233, Met 508, Tyr 118, Phe 380, Pro 230, Leu 121,Tyr 132, Phe 228, Ser 507, Tyr 64	**Site 1:** His 377, Phe 233, Met 508, Tyr 118, Phe 380, Pro 230, Leu 121, Thr 122, Tyr 132, Phe 228, Ser 507, Tyr 64 **Site 2:** Met 508, Pro 230, Phe 228, Ser 507, **Site 3:** His 377, Phe 233, Met 508, Phe 380, Pro 230, Ser 507, Tyr 64	No 'H' Bonds
MRA	Gly 303, Gly 307, Ile 304, Met 508, Phe 233, Phe 380, Tyr 118, Tyr 132, Leu 376, Thr 311	**Site 1:** Phe 233, Met 508, Tyr 118, Leu 376, Phe 380, Gly 303, Gly 307, Ile 304, Tyr 132, Tyr 118, Thr 311 **Site 2:** Met 508, Thr 311 **Site 3:** Phe 233, Met 508, Leu 376, Phe 380,	No 'H' Bonds
AT	Gly 307, Ile 131, Leu 121, Leu 376, Met 508, Phe 126, Phe 228, Phe 233, Phe 380, Thr 122, Thr 311, Tyr 118, Tyr 132, Val 509	**Site 1:** Gly 307, Ile 131, Leu 121, Leu 376, Met 508, Phe 126, Phe 228, Phe 233, Phe 380, Thr 122, Thr 311, Tyr 118, Tyr 132, Val 509 **Site 2:** Met 508, Phe 228, Thr 311 **Site 3:** Leu 376,, Met 508, Phe	No 'H' Bonds
ATA	Gly 307, His 310, Ile 304, Leu 121, Leu 376, Met 508, Phe 126, Phe 228, Phe 233, Thr 122, Thr 311, Tyr 118, Tyr 132, Val 509, Gly 303	**Site 1:** Gly 307, Ile 304, His 310, Leu 121, Leu 376, Met 508, Phe 126, Phe 228, Phe 233, Thr 122, Thr 311, Tyr 118, Tyr 132, Val 509, Gly 303 **Site 2:** His 310, Met 508, Phe 228, Thr 311, Val 509, **Site 3:** Leu 376, Met 508, Phe 233, Val 509\	No 'H' Bonds
FA	Gly 307, Ile 131, Leu 376, Phe 228, Thr 122, Tyr 132, Leu 121, Gly 303, Phe 126, Thr 311, Hem Ho1	**Site 1:** Leu 376, Leu 121, Gly 303, Gly 307, Thr 122, Tyr 132, Ile 131, Phe 228, Thr 311, Phe 126, **Site 2**: Phe 228, Thr 311 **Site 3:** Leu 376	No 'H' Bonds

Table 6: Docking analysis depicting residues in CYP51 of *Candida glabrata*, interacting with the ligands and presence of the residues in the reported binding sites

Ligand	Interacting residues of CYP51 (*C. glabrata*)	Presence of interacting residues in binding sites in CYP51 (*C. glabrata*) predicted by Metapocket	Ligand & protein atoms involved in H bonding
RA	Val 513, Tyr 127, Thr 510, Pro 380, Phe 385, Phe 242, Met 512, Leu 381, Leu 130, His 382, Gly 74, Tyr 73, Ser 511, Ser 383, Pro 239, Phe 509	**Site 1:** Val 513, Tyr 127, Thr 510, Pro 380, Phe 385, Phe 242, Met 512, Leu 381, Leu 130, His 382, Leu 130, Gly 74, Tyr 73 Ser 511, Ser 383, Pro 239, Phe 509 **Site 2:** Val 513, Met 512, Ser 511, Pro 239 **Site 3:** Pro 380, His 382, Phe 509	O; Pro 380 H O; His 382 N
MRA	Gly 311, Ile 140, Leu 381, Met 512, Phe 135, Phe 237, Phe 242, Tyr 141, Gly 315, Ser 511, Thr 319,Tyr 127, Hem	**Site 1:** Met 512 Leu 381, Ser 511, Gly 311, Ile 140, Phe 135, Phe 237, Phe 242, Tyr 141 Gly 315, Thr 319, Tyr 127 **Site 2:** Met 512, Ser 511, Gly 311, Phe 237, Gly 315, Thr 319	No 'H' bonds
AT	Leu 130, Leu 381, Met 512, Phe 237, Phe 385, Pro 239, Ser 383, Ser 511, Thr 319, Tyr 127, Val 513, Tyr 141, Leu 384, Thr 131	**Site 1:** Val 513, Tyr 127, Phe 385, Met 512, Leu 381, Leu 130, Ser 511, Pro 239, Phe 237, Tyr 141, Thr 319, Tyr 127, Thr 131, Leu 384, Ser 383, Leu 384 **Site 2:** Val 513, Tyr 127, Met 512, Ser 511, Pro 239 Phe 237 Thr 319	No 'H' bonds
ATA	Leu 130, Leu 381, Met 512, Phe 237, Phe 385, Pro 239, Ser 511, Thr 319, Tyr 127, Val 513, Leu 384, Thr 131, Gly 311, His 318, Ile 140, Phe 135, Phe 242, Thr 238, Gly 315	**Site 1:** Val 513, Tyr 127, Phe 385 Met 512, Leu 381, Leu 130, Ser 511, Pro 239, Phe 237, Phe 242, Gly 315, Thr 319, Tyr 127, Thr 131, Leu 384, Thr 131, Gly 311, His 318, Ile 140, Phe 135, Phe 242 **Site 2:** Val 513, Tyr 127 Met 512, Ser 511, Pro 239, Phe 237, Gly 315, Thr 319, Gly 311, His 318, Thr 238	No 'H' bonds
FA	Gly 311, Gly 315, Leu 381, Met 314, Phe 135, Phe 237, Thr 131, Thr 319, Ile 140	**Site 1:** Met 512, Leu 381 Gly 311, Ile 140, Phe 135, Phe 237, Gly 315, Thr 319, Met 314, Thr 131 **Site 2:** Met 512, Gly 311, Phe 237, Gly 315, Thr 319, Met 314	No 'H' bonds

Conclusion: Our present *in silico* studies establish the drug likeliness of rosmarinic acid (RA), alpha tocopherol (AT) and their derivatives. The docking analysis revealed the binding affinities of these compounds with the CYP51 of C.albicans and C.glabrata, being a key enzyme in the biosynthesis of ergosterol, an indicator of fungal biomass. Finally, these results indicate the anti-candidal effects of the selected ligands that may contribute in designing novel *in vivo* therapeutic strategies against *Candida* infections.

Acknowledgments: The authors are thankful to the Department of Biochemistry, Osmania University, Hyderabad for the computational facility to carry out the *in silico* studies and the Department of Biochemistry, Government City College, Hyderabad for the support during the Ph.D. studies of Vidya Chernapalli (VC).

Conflicts of interest: The authors declare no conflict of interest.

Author contributions: Conceptualization & supervision of the study, Karuna Rupula (KR); Execution, methodology & formal analysis, VC; Data curation by VC, Shyamala Chandra Rokkala (SCR) and Venkataiah Bhootham; Writing- review and editing by VC, SCR and KR.

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