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Green tea phytocompounds targets Lansterol 14-α demethylase against ergosterol biosynthesis in *Candida glabrata*

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ABSTRACT

Green tea is credited as one of the world's healthiest drinks with enriched antioxidants. It is known for its multi-beneficial health benefits against diabetes, blood pressure, hypertension, gastro-intestinal upset and is bestowed with significant antimicrobial potential. There are previous scientific evidence highlighting the antifungal potential of green tea and has identified it as a potential inhibitor of non-albicans *Candida* species. Lansterol 14-α demethylase (Erg 11) or CYP51 protein belongs to the cytochrome P450 monooxygenase (CYP) superfamily. Erg 11 is involved in ergosterol biosynthesis and has a significant role in azole drug resistance in *Candida glabrata*. The present study attempted to identify the inhibitory potential of green tea phytocompounds against inhibition of Erg 11 in *Candida glabrata* using bioinformatics tool viz., autodock vina software. Out of 15 green tea phytocompounds investigated, the study identified, Rutin (-10.5 kcal) Kaempferitrin (-9.4kcal), Epigallocatechin gallate (-10kcal), Epicatechin gallate (-8.7kcal), and Coumaroylquinic acid (-8.6kcal) acid as the potent phytocompounds which showed significant molecular interaction with Erg 11 in *Candida glabrata*. In attribution to the constant emergence of azole-resistant isolates, this preliminary analysis therefore, indicated the potential of green tea phytocompounds against inhibition of non-albicans *Candida* specific candidiasis. However, further, *in vitro* antimicrobial efficacy of these phytocompounds, the dose regime, drug likeliness, and cytotoxic analysis are required to be investigated and validated.

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INTRODUCTION

Candidiasis is a common fungal infection caused by yeasts from the genus *Candida*. The disease is associated with a diverse range of infections including mucosal, cutaneous, subcutaneous, and systemic mycoses. *C. albicans* was once considered to be majorly responsible for Candidiasis, however in the past few decades non-*albicans Candida* (NAC) species has been recognised to be the primary pathogens associated with increase incidences of Candidiasis. Epidemiological studies have shown that NAC species are responsible for 35-65% of all candidaemias in the general population (Presterl *et al.*, 2007; Arendrup, 2013).

C. glabrata includes 5-40% of all NAC species and accounts for about 15% to 35% mortality rate in susceptible individuals (Deorukhkar *et al.*, 2014; Rudramurthy *et al.*, 2017).

The limited count of specific antimicrobials against Candidiasis and emergences of increased drug toxicity in *C. glabrata* led to the investigation of future

alternatives for the significant potential antifungals that could prevent alarmingly increase outbreak of NAC infection (Du *et al.*, 2020).

Green tea is known for its multi-factorial health properties and is scientifically recognised as a possible antifungal drug (Anand *et al.*, 2015). As an urgent need to explore potent drug candidates for the development of novel medication against *C. glabrata*, the present study attempts to investigate the inhibitory potential of green tea phytocompounds against inhibition of Lansterol 14-α demethylase or Erg 11 protein *in silico*. Erg 11 protein is an essential protein of cytochrome P450 monooxygenase (CYP) superfamily that mediates crucial steps in ergosterol biosynthesis in *C. glabrata* and other fungus (Mishra *et al.*, 2007). Erg 11 (CYP51) is also a target site for azole drugs however, over-expression or point mutation in ERG11 has been attributed to the azoles resistance in *Candida* and other fungal strains (Geber *et al.*, 1995).

In silico docking analysis

In the present *in silico* study, we selected 15 phytocompounds present in green tea based on their reported antimicrobial activity against *Candida* spp. These phytocompounds were used as ligands for docking with the ergosterol biosynthesis proteins. Autodock vina (version 1.1.2) tool was used for assessing the molecular interaction based on binding energies. As a positive control, we used azoles (Fluconazole, Itraconazole, and ketoconazole) in the molecular docking analysis.

Preparation of ligands

The code of SMILES for all the aforementioned green tea phytocompounds and positive controls was procured from the online chemical database PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Table 1 and Table 2). The 2-D structures of the test ligands were retrieved by converting the SMILES code (.smiles format) into PDB (.pdb) format using chemical interconversion software Open Babel (v 2.3.1).

Retrieval of proteins-3D structures

The 3-D structure of Erg 11 (amino acid length-533) was retrieved in PDB format (using the PHYRE program (Protein Homology/analogY Recognition Engine), while its physico-chemical and functional characterization was achieved using Expasy's Protparam server (Gasteiger *et al.*, 2005). The 3D-protein homology model was generated and validated with a Ramachandran plot using the SWISS-MODEL interactive workspace (Arnold *et al.*, 2006).

Molecular docking

Molecular interaction between Erg 11 protein and the selected green tea phytocompounds as well as positive controls were analysed using Autodock vina software. The autodocking tool generated the binding energies of molecular interaction between all the green tea phytocompounds and Erg 11 PDB protein of ergosterol biosynthesis.

RESULTS AND DISCUSSION

In silico docking analysis

The Ramachandran plot depicted structural stability and showed confirmation of residues in the favorable region (Figure 1). A Ramachandran phi-psi plot for Erg 11 protein revealed 96.48% of residues in the allowed region (light grey), while 0.20% of the residues lay in the disallowed region (white) which indicated the preferable quality of protein model. The protein model showed 100% similarity with the target template (5jlc.1.A). The Q mean value of protein model was reliable, as depicted in the estimated Figure 2.

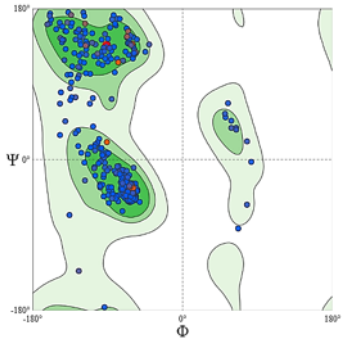

Figure 1: Ramachandran plot of Erg 11

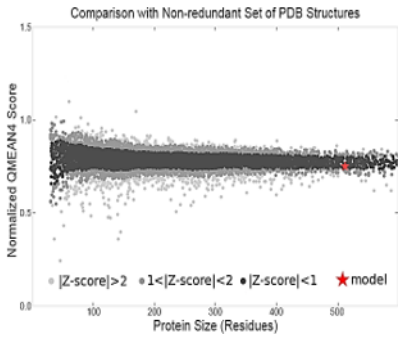

Figure 2: Q mean Z-score value of Erg 11

The *in silico* analysis of the green tea phytocompounds demonstrated significant interaction with the docked Erg 11 protein. The binding efficacy of the test ligands was assessed based on the binding energy of their molecular interaction and compared with the positive control. Among the 15 phytocompounds examined, Rutin (-10.5 kcal) Kaempferitrin (-9.4kcal), Epigallocatechin gallate (-10kcal), Epicatechin gallate (-8.7kcal), and Coumaroylquinic acid (-8.6kcal) were screened as the most active green tea

Table 1: Green tea Phytocompounds used as ligands

S.No.	Phytocompounds	PUBCHEM ID	SMILES code
1.	2,5-Dimethyl1-4-hydroxy-3-(2H)-furanone	14259114	OCC1OC(OC2=C(C)OC(C2=O)C)C(C(C1O)O)O
2.	B-ionone	26955	CC(=O)C=CC1=C(C)CCCC1(C)C
3.	Chlorogenic acid	348159	O=C(OC1CC(O)(CC(C1O)O)C(=O)O)C=Cc1ccc(c(c1)O)O
4.	Coumaroylquinic acid	53420248	O=C(OC1CC(O)(CC(C1O)O)C(=O)O)C=Cc1ccc(cc1)O
5.	Dihydroactinidiolide	27029	O=C1C=C2C(O1)(C)CCCC2(C)C
6.	Epicatechin (EC)	1203	Oc1cc2OC(c3ccc(c(c3)O)O)C(Cc2c(c1)O)O
7.	Epicatechin gallate (ECG)	367141	Oc1cc(O)c2c(c1)OC(C(C2)OC(=O)c1cc(O)c(c(c1)O)O)c1ccc(c(c1)O)O
8.	Epigallocatechin gallate (EGCG)	65064	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)OC(=O)C4=CC(=C(C(=C4)O)O)O
9.	Epigallocatechin (EGC)	1249	Oc1cc2OC(c3cc(O)c(c(c3)O)O)C(Cc2c(c1)O)O
10.	Gallic acid	370	OC(=O)c1cc(O)c(c(c1)O)O
11.	Kaempferitrin	12305415	Oc1ccc(cc1)c1oc2cc(OC3OC(C)C(C(C3O)O)O)cc(c2c(=O)c1OC1OC(C)C(C(C1O)O)O)O
12.	Myricetin	5281672	Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1cc(O)c(c(c1)O)O
13.	Pyrazine	9261	n1ccncc1
14.	Quercetin	5280343	Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O
15.	Rutin	5280805	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O

Table 2: Positive controls used in molecular docking study

S.NO.	Inhibitors (antibiotics/drugs)		PUBCHEM ID	SMILES CODE
1.	Azoles	Fluconazole	3365	C1=CC(=C(C=C1F)F)C(CN2C=NC=N2)(CN3C=NC=N3)O
		Ketoconazole	236076	CC(=O)N1CCN(CC1)C2=CC=C(C=C2)OCC3COC(O3)(CN4C=CN=C4)C5=C(C=C(C=C5)Cl)Cl
		Itraconazole	55283	CCC(C)N1C(=O)N(C=N1)C2=CC=C(C=C2)N3CCN(CC3)C4=CC=C(C=C4)OCC5COC(O5)(CN6C=NC=N6)C7=C(C=C(C=C7)Cl)Cl

phytocompounds which significantly showed binding with drug target Erg 11 (Table 3).

These findings are in agreement with our previous *in silico* investigation in which we highlighted the inhibitory effect of green tea phytocompounds like Kaempferitrin, Epigallocatechin gallate and Rutin against *Candida albicans* (Anand *et al.*, 2015). Based on the binding scores, rutin interacted with Erg 11 at the amino acid residues TRY-84, SER-95, GLY-251, ASN-284, TRY-288, ARG-314, PHE-318, and LEU-344 with the least binding energy of -10.50 kcal (Figure 3).

The binding affinity of the identified drug candidates showed significantly higher binding affinity in comparison to the azoles which are the recognised inhibitors of Erg 11 (Table 4). Erg 11 is a well-known azoles target and is associated with azoles resistance in *Candida* species linked with ERG 11 point mutation or over-expression (Zhou *et al.*, 2018). This essential protein mediates demethylation of the 14-α position of lanosterol to form ergosterol that maintains the permeability and fluidity of cell membrane essential for its growth and development (Zhang *et al.*, 2019). It is pertinent that inhibition of Erg 11 can reduce the cell membrane per-

Table 3: Binding energies of molecular interaction between ligands and Erg 11

Green tea phytocompounds (Ligands)	Binding energies (kcal)
Gallic acid	-6
Epicatechin	-6.5
Dihydroactinidiolide	-6.5
Pyrazine	-3.7
Chlorogenic acid	-7.8
Coumaroylquinic acid	-8.6
Quercetin	-7.8
Epigallate catechin	-7
Epigallocatechin gallate	-10
Myricetin	-8.4
β-ionone	-7.1
Epicatechin gallate	-8.7
Kaempferitrin	-9.4
Rutin	-10.5
2, 5- Dimethyl-4- hydroxy-3- (2H) – furanone	-5

Table 4: Binding energies of molecular interaction of positive controls and Erg 11

Inhibitors (antibiotics/drugs)		Binding energy
Azoles	Fluconazole	-7.62
	Ketoconazole	-8.24
	Itraconazole	-10.69

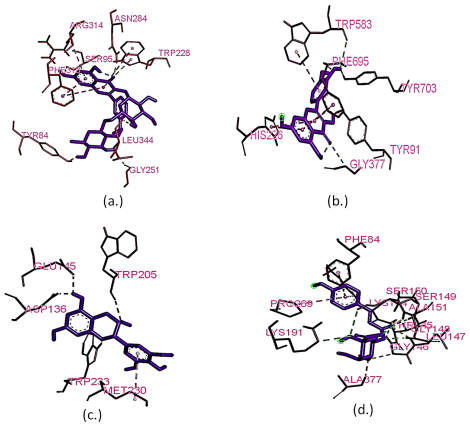

Figure 3: Molecular Docking images of interaction of Erg 11 and respective ligands; (a). Rutin (b). EGCG (c). ECG (d). Coumaroylquinic acid

meability of *Candida* sp. and could hamper their invasion and pathogenicity (Lv *et al.*, 2016; Villasmil *et al.*, 2020).

CONCLUSION

The present study highlighted the antifungal potency of green tea phytocompounds against inhibition of Lansterol 14-α- demethylase or Erg11 participating in ergosterol biosynthesis in *C. glabrata*. Based on the molecular docking studies, Kaempferitrin, Rutin, Epigallocatechin gallate, Epicatechin gallate and Coumaroylquinic acid were screened as the most active green tea phytocompounds and showed favourable binding with Erg 11 protein in ergosterol biosynthesis. In attribution to the constant emergence of azole-resistant isolates, this preliminary analysis is an attempt to explore alternative drugs for the inhibition of NAC species and thus further *in vitro* antimicrobial studies of these phytocompounds, the dose regime, drug likeliness, and cytotoxic analysis are required to be investigated.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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