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Computational analysis of phytocompounds with 1, 3 - β-D-Glucan synthase for antidermatophytic activity

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ABSTRACT

Skin diseases are the most common infection in humans as well as in animals caused by fungi like yeast, mould and dermatophytes. In this study, *in silico* analysis of 20 phytocompounds was carried out for their efficacy as antidermatophytic agents using the receptor 1, 3 - β -D-Glucan synthase. The 3D structure of the receptor obtained using Modeller9V8 was validated with Procheck, where Ramachandran plot showed 80.3% of residues in the most favoured region. The phytocompounds and the drugs Echinocandin B and Caspofungin were docked with 1, 3 - β -D-Glucan synthase using Glide. Though all the 20 compounds exhibited lesser energy than both Echinocandin B (-3.3Kcal/mol) and Caspofungin (-1.68 Kcal/mol), Quercetin-3-*O*-rutinoside exhibited very less energy (-11.56 Kcal/mol). Further, comparing to synthetic drugs, the entire compounds selected for this study showed high interaction with the modeled protein. Hence, the present study concludes that the efficacy of all phytocompounds used in this study act against dermatophyes and which will be very helpful to the researchers working in the area of dermatophye drug developments.

INTRODUCTION

People of all ages are affected by skin infections, the causative agents may be yeast cell *Candida albicans*, mold and dermatophytes of which the last said is keratinophilic (keratin degrading) fungi which invades keratin of skin, hair and nail (Weitzman and Richard, 1995). The preffix of the common name "Tinea" is based on the site of infection such as Tinea captitis (hair and scalp), Tinea cruris (groin), Tinea faciei (face), Tinea barabae (hairs and skin in the beard area), Tinea pedis (foot) and Tinea unguium (nail). Dermatophytosis treatment includes topical antifungal agents and synthetic antifungal drugs (Dixon and Walsh, 1996), which mainly falls into six main categories such as polyenes, azoles, allylamine and morpholine drugs, antimetabolite drugs, echinocandins and the other one is griseovolvin (Bennett, 2011). These classes of antifungal drugs have specific mode of action against the fungal cell wall where the polyenes such as amphotericin, nystatin and pimaricin interact with the sterols present in the cell membrane therefore to form channels that leads to leakage of small molecules from inside to outside of the cell wall (Warnock, 1991), azole based drugs which are classified as imidazoles (clotrimazole, miconazole and ketoconazole) and triazoles (itraconazole and fluconazole) inhibit cytochrome P_{450}-dependant enzymes (particularly C14-demethylase) involved in the biosynthesis of ergosterol (White *et al.*, 1998), from lanosterol which leads to the accumulation of toxic 14-α-methyl sterols and reduction of membrane-associated ergosterol thereby inhibiting the cell growth by altering the membrane properties and function (Groll *et al.*, 1998). Echinocandins such as anidulafungin, caspofungin and micafungin are water soluble lipopeptide molecules specifically target cell wall 1, 3-β –D-glucan synthase (GS) (Douglas, 2001). GS maintains the cell wall integrity which involves in the cell division and growth (Hector, 1993). Most of the treatment for skin infections with synthetic drug takes long time to show their effect and also with several side effects such as anorexia, constipation, headache, hepatitis, pruritis, and exanthema, inhibition of steroid hormone synthesis includes nausea, dizziness and gastrointestinal upset (Katsambas *et al.*, 1989; Grant and Clissold, 1989). As fungal cells are eukaryotes, most of the antifungal agents toxic to fungi are also toxic to the host. Hence antifungal agents are developed targetting cell wall components (Fig. 1) since cell wall is not shared by the mammalian cells, they are formed to be a good target for

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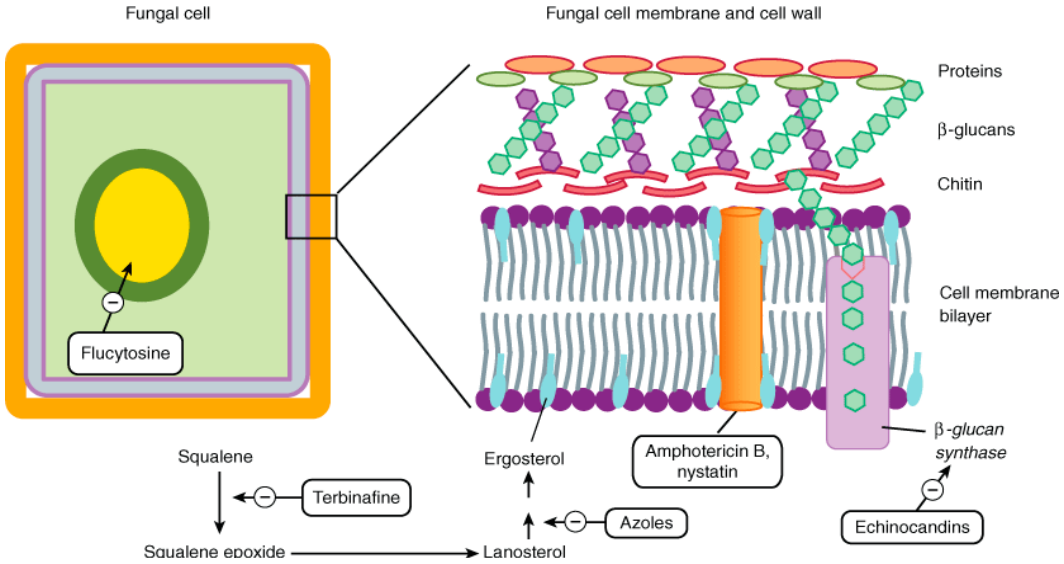

Fig. 1: Diagrammatic representation of the fungal cell wall targets and their antifungal drugs (Bennett JE. 2011).

antifungal drugs (Andriole and Bodey, 1994; Denning, 2003). Bowman and Free (2006) reported that 65 to 90% of the glucan are 1, 3-β –D Glucan. FKS1 and FKS2 gene encode 215-kDa, 217-kDa integral membrane proteins (Fks1p, Fks2p) are which alternating subunits with essential overlapping function of GS and thus disruption of these genes are lethal (Mazur *et al.*, 1995). Expression of FKS1 and FKS2 reveals that transcription of FKS1 is regulated in the cell cycle and predominates during growth on glucose, while FKS2 is expressed in the absence of glucose and is essential for sporulation (Douglas *et al.*, 1997). 1, 3-β –D Glucan is a major structural polymer of yeast and fungal cell walls and is synthesized from UDP-glucose by the multi-subunit enzyme 1, 3-β –D Glucan Synthase (GS) which has been selected as the target.

The phytocompounds were selected from various medicinal plants such as Vitexin, Agnuside, Negundoside and Aucubin from *Vitex negundo* (Niagariya *et al.*, 2010), Catechin, Kaempferol 3-*O*- β-D- glucoside, Kaempferol -3-*O*-rutinoside, Quercetin -3-*O*-rutinoside and Quercetin-3-*O*-β-glucoside from *Azadirachta indica* (Biswas *et al.*, 2002), Mangiferin from *Mangifera indica* (Wauthoz *et al.*, 2007), Apiin and Luteolin from *Lawsonia inermis* (Varghese *et al.*, 2010), Amaroswerin from *Swertia chirayita* (Joshi and Dhawan, 2005), Apigetrin from *Torenia fournieri* (Shindo *et al.*, 2008), Kaempferol from *Adhatoda vasica* (Ahmad *et al.*, 2009), Neoandrographolide from *Andrographis paniculata* (Chao and Lin, 2010), Nodakenetin from *Psoralea corylifolia* and Tembetarine from *Tinospora cordifolia* (Singh *et al.*, 2003), Aloin from *Aloe vera* (Dorai *et al.*, 2010) and Orientin from *Ocimum sanctum* (Udupa *et al.*, 2006).

The present study was designed 1). to model the 1, 3 – β –D-Glucan synthase and evaluate the modeled structure using Prochek 2) to find the active site regions of modeled protein 3) to select the phytocompounds from medicinal plants 4) to dock the selected phytocompounds with the modeled protein and computationally evaluate the efficacy of phyto-compounds against dermatophytes.

MATERIALS AND METHODS

Molecular Modeling

The sequence of 1, 3-β –D-glucan synthase protein was retrieved from Swiss-Prot database. Using Muster on line serve, the template was obtained and the 3D structure was modeled with Modeller9V8 and validated through SAVES. Active site residues were identified using Q-Site Finder.

RETRIEVAL OF PHYTOCOMPOUNDS AND SEMI-SYNTHETIC COMPOUNDS

The 3D structure of phytocompunds was retrived from PubChem database based on the antifungal activity of selected medicinal plants and synthetic compounds were retrived from Drug Bank database.

MOLECULAR DOCKING

Docking analysis was carried out for the modeled 1, 3-β-D-Glucan synthase (GS) with the selected phytocompounds and semi-synthetic drugs using Glide (a Schrodinger module).

RESULTS AND DISCUSSION

The dermatophytoses caused by keratinophilic fungi, dermatophytes, affect both animals and human beings. As the prolonged treatment of dermatophytoses and other fungal diseases with antifungal agents cause side effects, there is a need to find a drug with more potency than the existing ones. Moreover much attention has to be paid in discovering the drugs against fungal diseases as they are eukaryotes. The present antifungal agents such as caspofungin and echinocandin B, actidione, griseofulvin, fluconozole, itraconazole, ketoconazole target the cell wall, nucleic acids, sterol and other components of the fungal cell. The fungal cell wall components are very good targets for the development of antifungal drugs. Catalytic subunits of glucan

synthase (GS) are expressed during cell wall formation and FKS1 transcription is cell-cycle regulated and linked to cell wall remodeling (Denning, 2003). Disruption of FKS1 reduces glucan synthase activity as FKS gene products are associated with the catalytic subunit of GS (Douglas *et al.*, 1994). There is a report as FKS and RHO1 genes are highly conserved amongst fungi and are most required for cell viability (Beauvais *et al.*, 2001). Therefore the inhibition of 1,3-β-D-glucan synthase leads to disturb cell wall formation and prevents fungal growth. The 3D structure of this protein is unavailable. Hence, in this study, 3D structure of this protein is modeled using Modeler9v8 and validated using Procheck. The protein sequence of catalytic subunit of 1, 3-β-D-Glucan Synthase (Acc. No: B8XH77) retrieved from Swissprot consists of 240 amino acids. The template structures (1K47:A, 1NEX:A, 1CT9:A, 1KKH:A and 1YT3:A) with similar sequences for catalytic subunit of 1, 3-β -D-glucan synthase were identified using Muster and modeled using multiple template methods of Modeler9V8. The modeled protein was subjected to energy minimization using Swiss PDB Viewer and the energy value was 12507.987 Kcal/mol (Fig. 2). The RMSD value of modeled GS protein was 1.112 which was calculated using template structure (1K47:A).

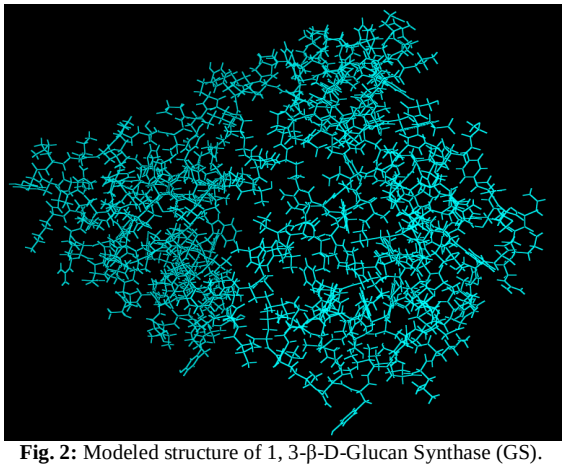

Fig. 2: Modeled structure of 1, 3-β-D-Glucan Synthase (GS).

The modeled protein structure was validated using Procheck and from the Ramachandran plot it was inferred that the modeled protein contain 80.3% of amino acid residues in the most favoured region, 5.2% in additional allowed region, 1.8% in general allowed region and only 2.7% of amino acid residues in disallowed region (Fig. 3). As the RMSD value is lower than 2.0 and more than 80% of the residues are in most favoured region, the modeled structure can be considered to be a good one. The active site residues of the modeled protein obtained using Q-site finder are LEU7, LEU9, PHE16, LEU21, ILE23, LYS26, SER27, TYR29, ALA30, SER32, ILE33 and PHE36. Mantle *et al.* (2001) reported that approximately one-third of all traditional medicines are for treatment of wounds or skin disorders, compared to only 1-3 % of modern drugs. The phytocompounds are selected on the basis of the antifungal activity of the plants and the 3D structures were retrieved from PubChem database and of the drugs Echinocandin B and Caspofungin from DrugBank database. The phytocompounds kampferol, quercetin (Yang *et al.*, 2001; Wang *et al.*, 2006), mangiferin (Ghosal *et al.*, 1977), agnuside (Mohandass *et al.*, 2010), orientin (De Campos *et al.*, 2005) and vitexin (Azzaz *et al.*, 2011) had antifungal activity and luteolin had antidermatophytic activity **(**Sartori *et al.*, 2003**).**

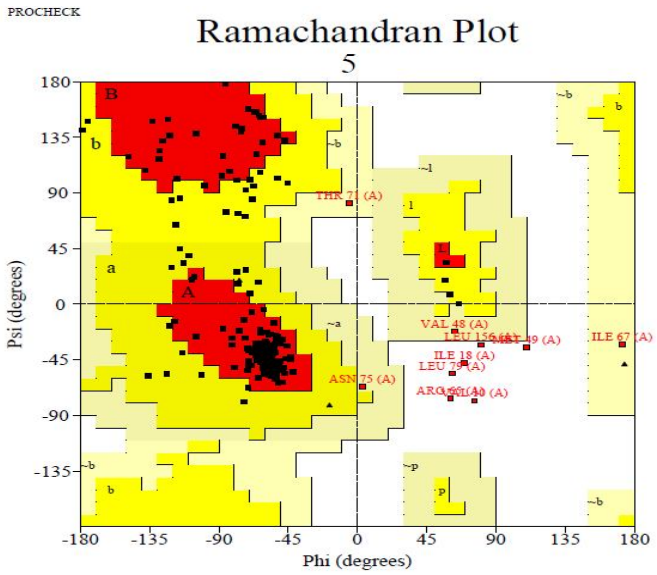

Fig. 3: Ramachandran plot for the Modeled target protein.

Table. 1: Docking results of semi-synthetic drugs with modeled 1, 3-β-D-Glucan Synthase (GS).

S.No.	Synthetic drug name	G.Score (kcal/mol)	Interacting Residues
1	Echinocandin B	-3.30	**ILE23**, GLN17, LYS20
2	Caspofungin	-1.68	**SER32**

Molecular docking studies were performed for modeled GS protein with the synthetic drugs (Table 1) and the selected phytocompounds (Table 2). The results were analyzed based on the interaction of H-bonds, interacting residues and G-Socre. The better interaction was selected by figuring out the minimum G-score. The same predictions were done for all the ligands and the results were analyzed. From the results, all the phytocompounds showed better interaction with GS protein than the drugs (Fig.4-Fig.7). Caspofungin and echinocandin B are the semisynthetic drugs which act as inhibitors for 1, 3-β-D-Glucan synthase and they were taken as standard to compare the efficacy of the phytocompounds. Among the 20 compounds, Quercetin-3-*O*-rutinoside gave the best interaction with the amino acid residues (THR 15, LYS 17, LYS 26, SER 27 and TYR 29) of GS protein with lowest energy level (-11.56 Kcal/mol) on comparison with the drugs and Tembetarine showed lowest interaction (-7.57 Kcal/mol) with the modeled protein. Further, all the compounds selected for this study showed better results than the drugs (Echinocandin B, -3.30 Kcal/mol; Caspofungin, -1.68 Kcal/mol). Quercetin-3-*O*-rutinoside had the best interaction with GS protein and the docked complex had low energy level and can be considered as potential inhibitor for 1, 3-β-D-Glucan synthase to treat Dermatophytosis.

Table. 2: Docking results of phytocompounds with modeled 1, 3-β-D-Glucan Synthase (GS).

S.No.	Compounds Name	G.Score (kcal/mol)	Interacting Residues
1	Quercetin-3-*O*-rutinoside	-11.56	THR15,LYS17, LYS26,SER27,TYR29
2	Mangiferin	-10.84	LYS26,SER27
3	Apiin	-10.82	THR15,TYR29
4	Agnuside	-10.54	GLN17,LYS20,ILE2,LYS26
5	Kaempferol-3-*O*-rutinoside	-10.35	THR15,LYS20,SER27
6	Amaroswerin	-10.25	LYS20,ILE23,LYS26
7	Apigetrin	-10.22	GLN17,LYS20, TYR29
8	Orientin	-9.58	LYS26,TYR29
9	Quercetin-3-*O*-β-D-glucoside	-9.35	TYR14,THR15, GLN17,LYS20, SER27
10	Negundoside	-8.89	TYR14,LYS26,SER2,ALA30,SER32
11	Aloin	-8.85	TYR14,LYS26, ALA30
12	Kaempferol 3-*O*-β-D-glucoside	-8.82	TYR14,LYS26, SER27,SER32
13	Vitexin	-8.62	LYS26
14	Luteolin	-8.62	SER27,TYR29,SER32
15	Catechin	-8.58	LYS26,TYR29
16	Kaempferol	-8.35	SER27,SER32
17	Neoandrographolide	-8.30	THR15
18	Aucubin	-8.30	THR15,ILE23, SER27
19	Nodakenetin	-8.20	SER32
20	Tembetarine	-7.57	TYR29

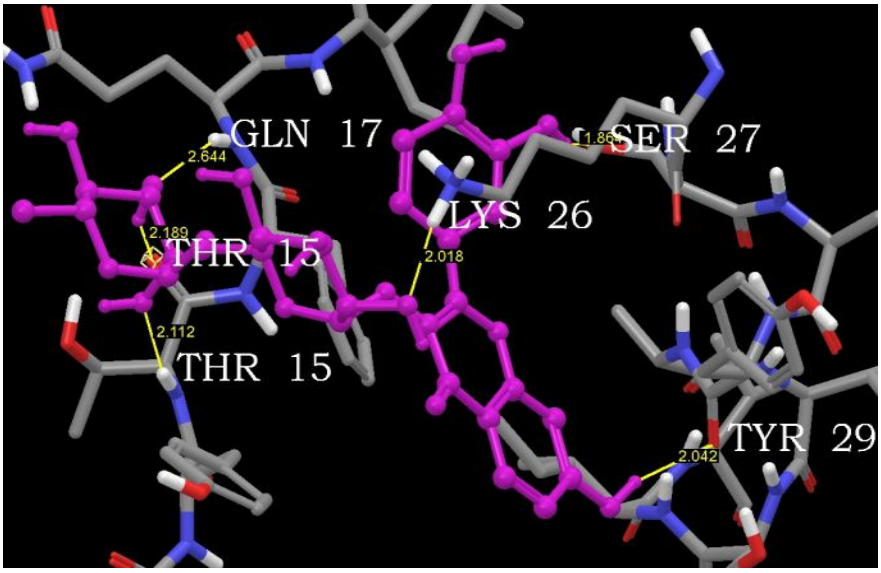

Fig.4: Interaction of Quercetin-3-*O*-rutinoside with 1, 3-β-D-Glucan Synthase (GS).

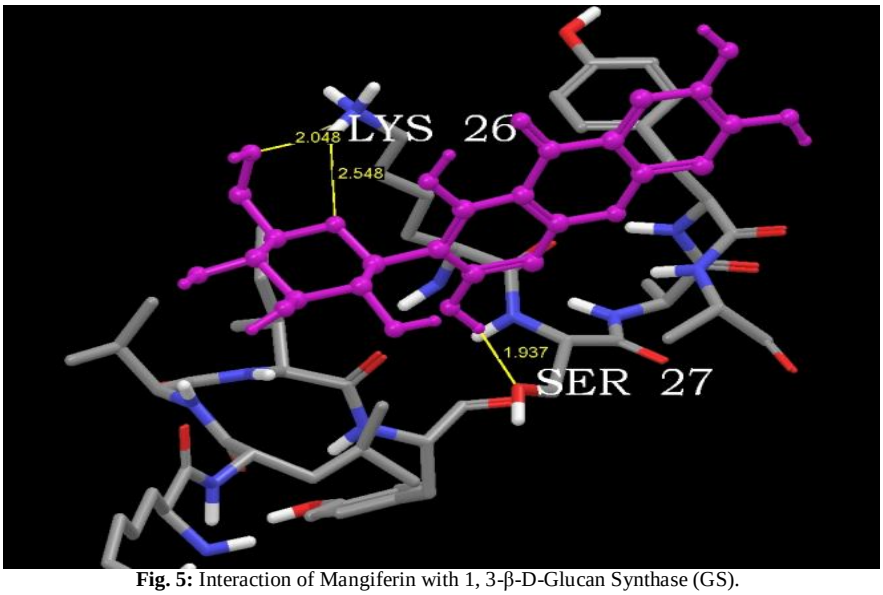

Fig. 5: Interaction of Mangiferin with 1, 3-β-D-Glucan Synthase (GS).

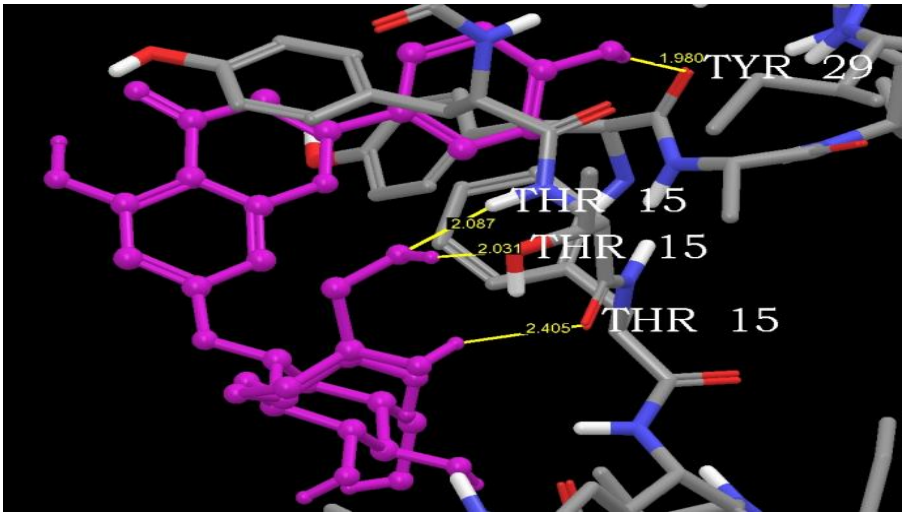

Fig. 6: Interaction of Apiin with 1, 3-β-D-Glucan Synthase (GS).

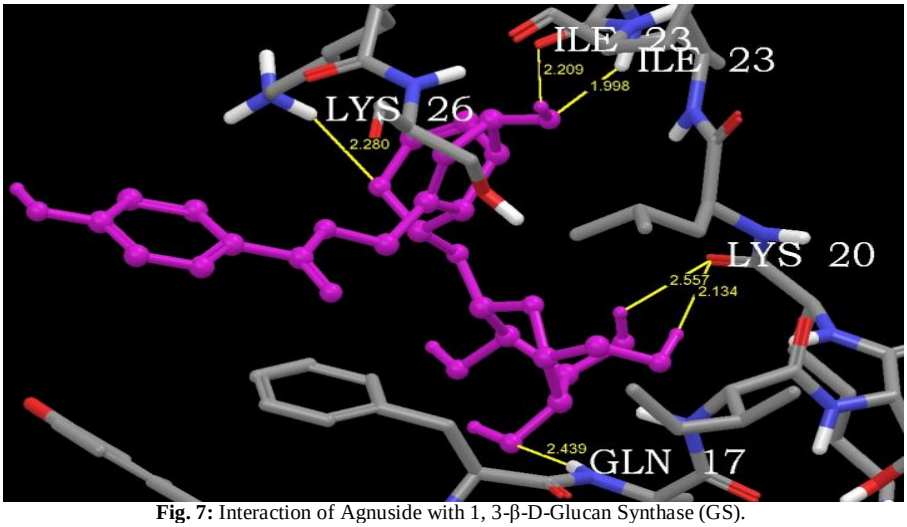

Fig. 7: Interaction of Agnuside with 1, 3-β-D-Glucan Synthase (GS).

CONCLUSION

The present study concludes that twenty phytocompounds selected for this study showed very good interaction with GS protein than the drugs Echinocandin B and Caspofungin. Thus, all the phytocompounds have ability to act as a potent inhibitor for 1, 3 - β -D-Glucan synthase. Of the 20 compounds and the drugs, Quercetin-3-*O*-rutinoside act as the best inhibitor for 1, 3 - β -D-Glucan synthase. For avoiding the side effects of synthetic / semisynthetic drugs, the results of the present study suggest that these 20 phytocompounds and mainly Quercetin-3-*O*-rutinoside can be considered as drugs or lead compounds to treat fungal infections particularly Dermatophytosis. Hence, this study will be very useful to the researchers working in the area of drug developments for Dermatophytoses.

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