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IN SILICO AND IN VITRO EVALUATION OF ANTI CANDIDAL ACTIVITY OF SELECTED MEDICINAL PLANTS

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ABSTRACT

Candida spp. once a commensal microbe becomes clinically important pathogen since it is the causative for several types of infection, ranging from superficial infections such as vulvovaginal candidiasis, esophageal or oropharyngeal candidiasis, to life-threatening invasive disorders. Medicinal plants being our traditional mean of treatment, the phytoconstituents of the plants *Acalypha indica, Aloe barbadensis, Andrographis paniculata, Argemone mexicana, Azadirachta indica* and *Vitex negundo* in the present study had been screened using molecular docking studies. The compound mauritianin from *Acalypha indica* showed least G.score value of -11.87 Kcal/mol for exo-β-1,3 glucanase, therefore, the methanol extract of *A. indica* was quantified for the presence of glycosides using HPTLC technique which indicated the higher presence of glycosides. Moreover, the anti-candida activity of the methanol leaf extract of *A. indica* also observed to inhibit the growth of *Candida albicans* significantly. Mauritianin, a glycoside might be reasoned for its efficient activity against *C. albicans.* Further studies to identify and isolate the bioactive principle from the *A. indica* extract have to be determined.

Keywords: Exo-β-1,3- glucanase, Molecular docking analysis, HPTLC, Candida albicans, Disc diffusion method.

Introduction

Candida species are referred as opportunistic fungi in the early period, however, lately these species becomes clinically important in parallel to the increase in number of immune compromised patients and the advances in intensive medical care (Oliveira et al., 2016). It is reported that *C. al bi cans* is third most common isolate as bloodstream pathogen and second most from blood cultures in hospitals (Ellis, 2002) and they are leading cause for mortality in different locations (Bhakshu et al., 2016).

Among 17 different Candida species, 90% of invasive infections are caused by *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis and C. krusei* (Pfaller et al., 2007). Treating the candidal infections include difficulties like fungal resistance and biofilm formation, are the major factors necessitate the search for new targets and antifungal agents (Sardi et al., 2011). At present era, searching for novel and effective drugs from natural sources that too probing the medicinal plants is the criteria being followed universally. Even WHO (World Health Organization) has reported that more than 80 % of the world's population relies on traditional herb medicines for their primary health care (Anant and Jaya, 2013). Moreover relying on plant source is considered as safe and effective (Doddanna et al., 2013). The present study also aims at screening the phytoconstituents of *Acalypha indica, Aloe barbadensis, Andrographis paniculata, Argemone mexicana, Azadirachta indica and Vitex negundo* for anti-candidal activity, where the study is designed to predict the binding efficiency through docking studies followed by *in vitro* analysis for the plant producing significant results *in silico.* Here, the chosen fungal target is exo-β-(1,3)-glucanase, where its importance in inducing hyphal formation of *C. albicans* was studied by Xu et al. (2013). *In vitro* analysis focuses on anti candidal activity and HPTLC profiling for the methanol extract of plant.

MATERIALS AND METHODS

In silico analysis

The 3D structure of protein target exo-β-(1,3)-glucanase was retrieved from the protein data bank (PDB ID: 1EQP). The phytochemical compounds of the selected plants were retrieved from the PubChem database. The compounds were predicted for the ADME-Toxicity through QikProp module of Schrodinger software. The active site for the protein was predicted using the LIGSITE and docking study was carried out in Glide module (Sathish et al., 2015).

In vitro analysis

Collection and preparation of plant material

The fresh leaves of *Acalypha indica* was collected from papanaickenpalayam, Coimbatore District, Tamilnadu, India during April, 2015. The plant was authenticated at Botanical Survey of India, Tamil Nadu Agricultural University (TNAU), Coimbatore (Ref no. BSI/SRC/5/23/2016/tech). The collected plant leaves are washed, shade dried and grained into coarse powder. Percolation technique

using methanol solvent was followed for extraction and crude extract was obtained for further analysis.

High Performance Thin Layer Chromatography (HPTLC)

The High performance thin layer chromatography (HPTLC) is an ideal tool for the identification for the presence of higher quantity of glycoside in the crude methanol extract of Acalypha indica leaf and compared with the standard. The plant sample was centrifuged at 3000rpm for 5mins and 2 µl of sample was loaded on 2 x 10 Silica gel 60F254 TLC plate of 5mm band length using Hamilton syringe (CAMAG LINOMAT 5). The plates were developed in TLC twin trough developing chamber with respective solvent Ethyl acetate-Ethanol-Water (8:2:1.2) as mobile phase up to 90mm. The developed plate was dried and kept in Photo-documentation chamber (CAMAG REPROSTAR 3) at visible light, UV 254nm and UV366nm. The Peak table, Peak display and Peak densitogram were noted. The Brown, Brownish Yellow colored zones at visible light mode and fluorescent yellow zones at UV 366nm mode were observed in the given standard and sample track, which confirmed the presence of glycoside in the given the sample.

Antifungal Activity

The methanol extract of plant leaves was tested for antifungal activity against the organism *Candida albicans* using disk diffusion method in an aseptic condition (Nagalingam et al., 2015). The filter paper discs were loaded with extracts and allowed to dry. The medium used was Saboraud Dextrose Agar (SDA) and a swab of *Candida albicans* (0.5 McFarland turbidity) was aseptically inoculated on petridishes and the prepared discs were placed. The anti candidal activity of the extract was compared with the standard drug ciprofloxacin (CIP), cefotaxime (CTX) of 30 mg concentration by measuring the zone of inhibition.

RESULTS

In silico Analysis

The interactions of phytochemical compounds with exo-β-(1,3) glucanase were tabulated along with the G-Score, number of hydrogen bonds, bond length and the interacting residues (Table 1). Among the plant compounds mauritianin from *A. indica* scored least G.score value of -11.87 Kcal/mol and formed 8 hydrogen bonds of bond length 1.5 to 2.7 Å (Fig.1).

Table 1: Interactions of selected plant compounds with Exo -β - (1, 3)-glucanase

S.No.	Ligands name	Interacting Residues	Bond length (Å)	No. of bonds	G-Score (Kcal/mol)
Synthetic drug					
1	Azaserine	ASN-146 (H-O)	2.0	4	-7.73
		GLU-27 (O-H)	1.9		
		ASP-145 (O-H)	2.3		
		LEU-304 (O-H)	2.0		
Acalypha indica					
1	Inositol	GLU-292 (O-H)	2.1	7	-7.75
		GLU-192 (O-H)	1.7		
		ASN-146 (H-O)	2.2		
		GLU-27 (O-H)	1.7		
		TYR-29 (H-O)	1.9		
		LEU-304 (O-H)	2.3		
		LEU-304 (O-H)	2.4		
2	2-Methyl anthraquinone	ASP-145 (O-O)	2.9	2	-5.62
		ASP-145 (H-O)	2.1		
3	n-Octacosanol	ASN-146 (H-O)	2.0	3	-3.7
		GLU-27 (O-H)	2.2		
		GLU-27 (O-H)	1.9		
4	Hesperitin	ASN-146 (H-O)	2.0	4	-7.77
		ASN-146 (H-O)	2.6		
		GLU-27 (O-H)	1.8		
		LEU-304 (O-H)	1.8		

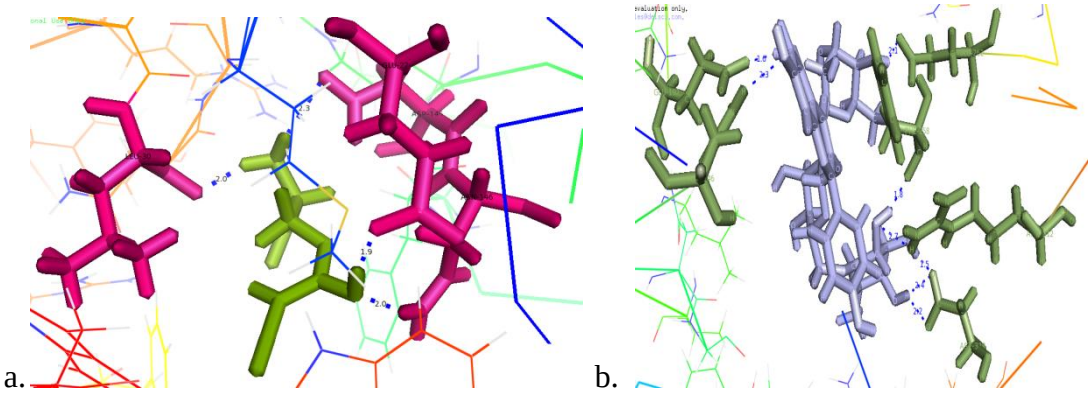
5	Quebrachitol	LEU-304 (O-H)	1.8	6	-7.72
		LEU-304 (O-H)	1.8		
		TYR-29 (H-O)	2.0		
		ASP-145 (O-H)	1.8		
		GLU-27 (O-H)	1.7		
		ASN-146 (H-O)	2.5		
6	Quercitrin	GLU-292 (O-H)	2.4	8	-11.26
		GLU-292 (O-H)	1.9		
		LEU-304 (O-H)	1.8		
		TYR-29 (H-O)	2.3		
		ASP-145 (H-O)	2.3		
		ASP-145 (O-H)	1.6		
		ASP-145 (O-H)	1.7		
		ASN-146 (H-O)	2.2		
7	Nicotiflorin	TYR-153 (H-O)	1.8	6	-10.8
		ASP-151 (O-H)	2.2		
		GLU-27 (O-H)	2.6		
		TYR-29 (H-O)	2		
		ASN-305 (O-H)	1.8		
		LEU-304 (O-H)	2.8		
8	Clitorin	TYR-255 (O-H)	2.0	6	-11.37
		GLU-262 (O-H)	2.0		
		GLU-262 (O-H)	2.0		
		GLU-192 (O-H)	2.3		
		GLU-192 (O-H)	1.6		
		ASP-145 (O-H)	1.7		
9	Mauritianin	GLU-262 (O-H)	2.1	8	**-11.87**
		GLU-192 (O-H)	1.6		
		ASN-146 (O-H)	2.3		
		PHE-258 (O-H)	1.8		
		ARG-312 (O-H)	2.7		
		ASP-318 (O-H)	1.5		
		ASP-318 (O-H)	2.4		
		ASP-318 (O-H)	2.2		
Aloe barbadensis					
10	Emodin	ASP-145 (O-O)	3.1	2	-5.46
		ASP-145 (O-H)	2.6		
11	Anthranol	GLU-27 (O-H)	2.1	2	-6.17
		ASN-146 (H-O)	2.1		
Andrographis paniculata					
12	Vanillic acid	ARG-312 (H-O)	1.7	4	-4.84

		ARG-309 (H-O)	2.2		
		ASP-145 (H-O)	2.1		
		ASP-145 (O-H)	2.2		
13	Quercetin	TYR-29 (H-O)	2.6	8	-9.39
		TYR-29 (H-O)	2.4		
		ARG-312 (H-O)	2.0		
		ARG-309 (H-O)	2.6		
		ASN-146 (H-O)	2.7		
		ASP-145 (O-O)	2.9		
		ASP-145 (H-O)	2.0		
		ASP-145 (O-H)	1.6		
14	Apigenin	GLU-27 (O-H)	1.7	3	-7.95
		TYR-29 (H-O)	2.1		
		ASP-145 (O-H)	1.7		
15	Andrographolide	LEU-304 (O-H)	1.9	2	-7.19
		ARG-312 (H-O)	1.8		
16	α-Sitosterol	TYR-153 (H-O)	2.2	1	-4.78
17	Iso-andragrapholide	GLU-292 (O-H)	1.7	3	-6.33
		ASN-146 (H-O)	2.7		
		TYR-29 (H-O)	2.1		
18	Deoxy-andrographolide	ASN-146 (H-O)	1.8	4	-6.69
		ASN-305 (O-H)	2.6		
		ARG-312 (H-O)	1.8		
		TYR-29 (H-O)	2.2		
19	Cosmosin	ARG-312 (H-O)	2.4	2	-4.3
		ASP-145 (O-H)	2.1		
20	Andrographiside	ARG-309 (H-O)	2.0	8	-7.95
		ARG-309 (H-O)	2.5		
		ARG-309 (H-O)	2.6		
		ASN-305 (O-H)	1.8		
		TYR-29 (H-O)	1.9		
		GLY-143 (O-H)	2.0		
		ASP-151 (O-H)	1.6		
		GLU-292 (O-H)	1.7		
Argemone mexicana					
21	Berberin	ASP-145 (H-O)	2.4	2	-7.74
		TYR-29 (H-O)	2.2		
22	Cysteine	GLU-292 (O-H)	2.4	5	-2.89
		TYR-255 (H-O)	2.7		
		TYR-29 (O-H)	2.5		
		GLU-27 (O-H)	2.3		

		ASN-146 (H-O)	2.1		
23	L-Phenylalanine	GLU-192 (O-H)	2.2	2	-4.8
		GLU-292 (O-H)	2.9		
24	Higenamine	ASP-145 (H-O)	1.9	3	-6.84
		ASP-145 (O-H)	1.9		
		GLU-192 (O-H)	1.7		
25	(-)-Argentamine	ASP-145 (H-O)	2.2	4	-8.82
		ASP-145 (O-H)	2.2		
		GLU-27 (O-O)	3.0		
		TYR-29 (H-O)	2.1		
Azadirachta indica					
26	Catechin	GLY-143 (O-H)	2.2	4	-7.83
		ARG-309 (H-O)	2.0		
		GLU-292 (O-H)	2.1		
		GLU-192 (O-H)	1.9		
27	Epicatechin	GLU-292 (O-H)	2.1	4	-7.83
		GLU-192 (O-H)	1.9		
		GLY-143 (O-H)	2.2		
		ARG-309 (H-O)	2.0		
28	Nimbolide	ARG-312 (H-O)	2.2	1	-5.95
29	Nimbin	ARG-309 (H-O)	2.6	2	-3.91
		ARG-309 (H-O)	2.0		
30	Meliacin	ARG-309 (H-O)	2.5	1	-3.27
31	Nimbidinin	GLY-143 (O-H)	2.3	2	-7.47
		TYR-29 (H-O)	1.9		
32	Azadirachtin	ARG-312 (H-O)	1.9	2	-4.31
		ASP-151 (O-H)	1.9		
33	Salanin	ARG-312 (H-O)	1.8	2	-4.04
		ASP-145 (H-O)	2.4		
34	Gedunin	ARG-312 (H-O)	2.5	2	-3.67
		ARG-309 (H-O)	2.5		
Vitex negundo					
35	Nerolidol	TYR-29 (O-H)	1.9	2	-1.72
		GLU-27 (O-H)	1.8		
36	Agnuside	ARG-309 (O-H)	2.8	7	-8.76
		ASP-145 (O-H)	2.5		
		GLU-292 (O-H)	2.7		
		TYR-255 (O-H)	2.4		
		TYR-255 (H-O)	2.3		
		TYR-255 (H-O)	2.7		
		ASP-145 (H-O)	2.0		

37	Vitexin	LEU-304 (O-H)	1.9	4	-10.25
		GLU-27 (O-H)	2.6		
		ASN-146 (H-O)	1.9		
		GLU-192 (O-H)	1.7		
38	Vitrofolal	GLU-192 (O-H)	1.8	5	-8.95
		ASN-146 (H-O)	2.7		
		GLU-27 (O-H)	2.0		
		ARG-312 (H-O)	1.9		
		TYR-29 (H-O)	2.1		
39	Vitedoamine	ASN-305 (O-H)	2.3	3	-7.84
		TYR-29 (H-O)	2.0		
		GLU-27 (O-H)	1.7		
40	Vitedoin	TYR-29 (H-H)	2.6	4	-9.59
		LEU-304 (O-H)	1.6		
		ASN-146 (H-O)	2.2		
		GLU-192 (O-H)	2.2		

Fig. 1: Screenshot of interactions with Exo -β - (1, 3)-glucanase a. Synthetic drug- Azaserine; b. Mauritianin of *Acalypha indica*



Based on the docking analysis, the plant *A. indica* was further evaluated through *in vitro* techniques.

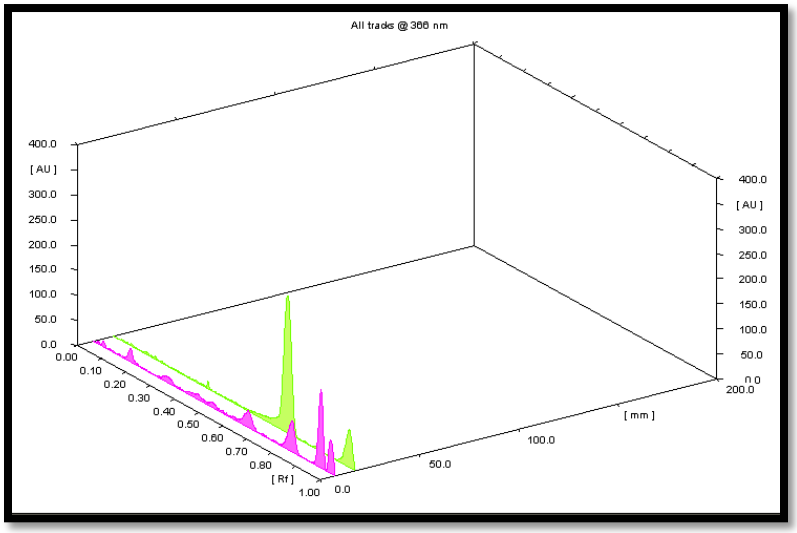
HPTLC Analysis

HPTLC profiling confirms the presence of glycosides and the corresponding Rf values, peak area were recorded (Table 2). The 3D display of chromatograms for both plant extract and the standard were shown (Fig.2).

Table 2: HPTLC Profiling of *Acalypha indica* extracts

Peak	R_f	Height	Area	Assigned substance
1	0.01	43.6	167.4	Unknown
2	0.04	11.8	121.7	Unknown
3	0.16	47.6	821.8	Glycoside 1
4	0.32	12.9	388.0	Glycoside 2
5	0.64	98.3	3175	Glycoside 3
6	0.82	79.4	2258.7	Glycoside 4
7	0.94	136.7	2482.0	Glycoside 5
8	0.72	470.8	12848.7	Glycoside standard

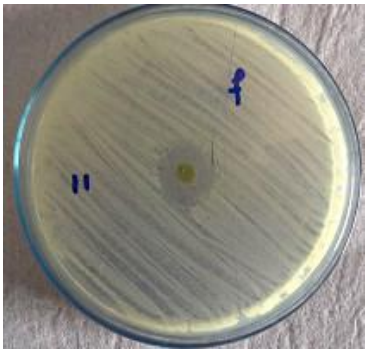
Fig.2: 3D Display of Peak densitogram of HPTLC chromatogram



Anticandida Acivity

The anti candida activity (Fig.3) showed zone of inhibition (ZI) of 17 mm for plant extract and 30 and 19 mm for CIP and CTX, respectively.

Fig. 3: Anti candida activity a. Methanol extract of *Acalypha indica* b. Negative control c. Positive control (CIP; CTX)

a. 

b. 

c. 

Discussion

In *Candida albicans*, the Exo-β-1,3-glucanase strongly favors β-1,3-linkages through its hydrolase and transglycosidase activities in β-D-glucan metabolism and morphogenesis (Nakatani *et al*., 2014). The structure of the protein was discussed by Cutfield *et al*., (1999) whereas the interaction of 2-fluoroglycosylpyranoside and a transition state analogue, castanospermine with glucose binding residues

Glu192 and Glu292 (Chambers *et al*., 1993; Mackenzie *et al*., 1997). The conserved residues Arg92 (β2 strand), His135 (β3), Asn191 (between β4 and α4), His253, Tyr255 (end of β6), Trp363 (end of β8) forms a distinctive pocket. In addition, the residues from loop 1, 3 and 7 contribute as the sides of the active site pocket.

The plant compounds observed to interact mostly with Glu27, where the residue is referred to serve as a dominant hydrogen-bonding in the exo-glucanase inhibition. The residue Tyr255 interacted with clitorin of *Acalypha indica*, cysteine of *A. mexicana* and agnuside of *V. negundo*. Few compounds in the selected plants were found to interact with Leu304, Asn146 and Tyr29 which are located in the loops of 7, 3 and 1, respectively. The compounds vitexin and vitedoin from *V. negundo*, inositol and quebrachitol from *Acalypha indica* interacted with the above said residues and also with the Glu192. Moreover, the interaction with the residues Glu192 and Glu292 indicated that the compounds interact with catalytic site of the protein.

Novel synthetic peptide, KU3 has lower MIC (8 and 16 mg/ml for different strains SC5314 and ATCC 90028) for *C. albicans* which had higher binding affinities for exo-β-1,3 glucanase forming hydrogen bonds with Glu27, Tyr29 and Glu372 and also electrostatic interactions with Asp145, Asn191, Glu192, Gly291, Gly292, Asn299 and Asp318 where in specific Glu192 and Glu292 are catalytic sites (Lum *et al*., 2015). In the present study, mauritianin from *Acalypha indica* had single interaction with Glu262, Glu192, Asn146, Phe258, Arg312 and bonds with Asp318 with a G.score of 11.87 Kcal/mol.

The plant *Acalypha indica* has been reviewed as one of the alternative medication for Candidiasis (Tariq et al., 2015) as well as the maximum antifungal activity was reported for *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus flavus* (Suresh et al., 2009). Previous reports indicated that chloroform extract to inhibit the *C. albicans* as equivalent to ketoconazole and fluconazole (Somchit et al., 2010). Here, the methanol extracts had 17mm ZI against *C. albicans* which is less compared to CIP and CTX. Also mauritianin, a glycoside molecule was observed with significant G.score where the HPTLC quantification indicated its presence in the methanol extract of *A. indica* leaves (Table 2). An alkyl glycoside potentially inhibited the growth of both laboratory and clinically isolated strains of *C. albicans* (Klunda et al., 2016). Additionally, daucosterol, a β-sitosterol glycoside induced the protective Thi immune response against disseminated Candidiasis in mice which seems to be advantage for the host to develop and combat the Candidiasis (Lee et al., 2007). Therefore, the effect of *A. indica* leaf methanol extract against *C. albicans* might be due to the presence of glycosides as major consitutents. Further studies could focus on determining and isolating the bioactive principle of the anti-candidal activity from *Acalypha indica* methanol extract.

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