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Research Article

In silico approach of receptor-ligand binding and interaction: Established phytoligands from Tagetes errecta Linn. against β -glucosidase receptor

Madhumita Lahiri¹, Partha Talukdar^{1*} and Soumendra Nath Talapatra²

- ¹Department of Botany, Serampore College, University of Calcutta, William Carey Road, Hooghly, West Bengal, India
- ²Department of Biological Science, Seacom Skills University, Kendradangal, Shantiniketan, Birbhum 731236, West Bengal, India

ABSTRACT

The medicinal plant, *Tagetes errecta* Linn. is a common ornamental plant and leaves of this plant are containing phytochemicals (volatile oil) that inhibit the growth of bacteria, fungi and known natural antimicrobial agents. The objective of the present study was to detect receptor-ligand binding energy and interaction through molecular docking for phytoligands established in the leaves of *T. errecta* against β-glucosidase receptor (PDB ID: 3AHZ). Molecular docking was performed by using PyRx (Version 0.8) for the structure-based virtual screening and visualized the interaction in the molecular graphic laboratory (MGL) tool (Version 1.5.6). Among 25 phytochemicals and 2 synthetic compounds (Carbendazim and 2-Amino-2-hydroxymethyl-propane-1,3-diol), binding energy value was obtained highest in Bicyclogermacrene (-6.4 Kcal/mol) and lowest in Octanol (-4.4 Kcal/mol) and Carbendazim and 2-Amino-2-hydroxymethyl-propane-1,3-diol) showed -6.7 Kcal/mol and -3.5 Kcal/mol and all of these showed no hydrogen bonding. The binding interaction of target protein with this phytocompound found binding at the mouth of the active site may be treated as competitive inhibitor. In conclusion, phytocompound Bicyclogermacrene can be alternative of synthetic fungistatic compound as per binding energy value and interaction. It is suggesting further pharmacological and toxicological assay with this phytocompound after isolation from ornamental plant (*T. errecta*).

Keywords: Tagetes errecta; Phytoligands; β-glucosidase receptor; Molecular docking; Receptor-ligand binding; In silico study

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*Address for Correspondence:

Partha Talukdar, Assistant Professor, Department of Botany, Serampore College, University of Calcutta, William Carey Road, Hooghly, West Bengal, India

INTRODUCTION

Among various ornamental plants, *Tagetes errecta* Linn. commonly known as marigold found in the tropics. From past to recent study, it was reported that the phytochemicals in the parts of this plant are antibacterial, antifungal, insecticidal, etc. in nature. According to Gupta, *Tagetes errecta* has antifungal effect on *Alternaria solani, Rhizoctonia solani, Rhizoctonia bataticola, Colletotrichum gloeosprioides,* Fusarium *oxysporum f.sp. pallidoroseum, Phoma sorghina, Sclerotium rolfsii*, etc. after usage of crude, boiled, powdered and ethanol extract of leaves, which is still unclear that single or combination of phytochemicals is acting as lead compound(s). Also, it is not possible to isolate each phytocompound from leaf and conduct experiment to detect fungistatic activity that may require laboratory expanses, long duration, etc.

From past, computer-based receptor-ligand binding as a suitable approach for structure-based drug screening and exact phytocompound or combinations of few phytochemicals can be predicted within a few hours by using

molecular docking and interaction.⁶ On the other hand, the molecular docking tool is used to predict the interaction between a small molecule (ligand) and a macromolecule (protein) that describes the behavioural characterization of small molecules in the binding site of target receptor.⁶⁻¹⁰

It is an interesting research that natural compounds not only use in pharmaceuticals while can use for insecticides, pesticides, biofertilizers, etc. Among these, fungistatic is an important compound to prevent various fungal infection in which researchers are showing interest to inhibit the multiplication of fungal activities. Among several enzymes in fungi, β -glucosidase enzyme is most effective for the hydrolysis of cellulose to glucose monomer. 11 Moreover, fungal resistance to existing fungicides pose serious threat to prevent pathogenicity 12 but plant-based natural compound may be suitable for inhibitory activities. 13

The objective of the present computational prediction was to detect suitable receptor-ligand binding energy and molecular interaction through molecular docking approach for phytoligands established in *T. errecta* and synthetic

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compound as Carbendazim and 2-Amino-2-hydroxymethyl-propane-1,3-diol against β -glucosidase receptor (PDB ID: 3AHZ) for fungistatic compound.

MATERIALS AND METHODS

Selection of protein (receptor)

The crystal three-dimensional (3-D) structure of protein of β-glucosidase receptor (PDB ID: 3AHZ) was retrieved from European protein (http://www.ebi.ac.uk/pdbe/). al.14 Jeng et have experimented and deposited the X-ray diffraction crystallographic 3-D structure of the β -glucosidase attached with two bound molecules as glycerol 4002 and 2-amino-2hydroxymethyl-propane-1,3-diol 4001 of 1.34Å resolution. The 3-D ribbon structure is exhibited in Fig 1 after visualizing in MGL Tool developed by The Scripps Research Institute.15

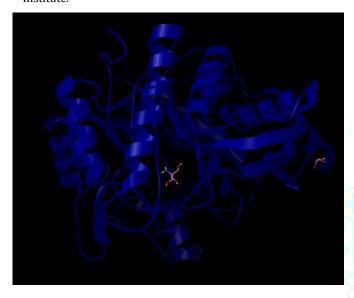


Figure 1: Three-dimensional (3D) ribbon structure of β-glucosidase (PDB ID: 3AHZ) attached with ligands (line structure) as 2-Amino-2-hydroxymethyl-propane-1,3-diol (TRS) 4001 and glycerol (GOL) 4002

Selection of phytochemicals (ligands)

The selection of phytochemicals (ligands) of *T. errecta* were done from the literatures as per antifungal properties. ¹⁶⁻¹⁸ In the present study, established 25 phytochemicals of *T. errecta* reported as natural compounds (volatile oil) present in leaf ¹⁶ and two synthetic compounds as fungicide and protein inhibitory molecule were taken. The Canonical SMILES of these compounds were taken from the PubChem database (www.ncbi.nlm.nih.gov/pubchem) and .pdb file of each phytochemical was obtained from CORINA online server (www.mn-am.com/) after inserting SMILES string in appropriate place. The plant is depicted in Fig 2 and 3-D structure of all the selected ligands are depicted in Fig 3.



Figure 2: Ornamental plant (T. errecta Linn.)

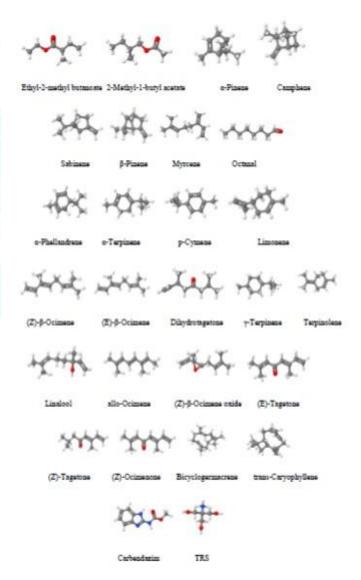


Figure 3: 3-D structure of phytoligands and synthetic compounds from CORINA server

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Study of molecular docking and interaction

The docking was carried out by a virtual screening method through PyRx software (Virtual Screening Tool, Ver 0.8) developed by Trott and Olson.¹⁹ The molecular docking was visualized the output .pdbqt file by using MGL tool, developed by The Scripps Research Institute¹⁵ and the results of 3-D structure were rendered by using MGL Tools. Docking of 25 phytochemicals and 2 synthetic ligands with βglucosidase (PDB ID: 3AHZ) were analysed to detect suitable binding energy value. The phytoconstituents and synthetic compounds with the β -glucosidase protein (receptor) to identify the residues involved in each case of receptor-ligand interactions. The docking site on this target protein was expressed by forming a grid box with the dimensions of X: 60.5828, Y: 53.8248 and Z: 64.4599 Å, with a grid spacing of 0.375 Å, centered on X: -29.0908, Y: 87.7936 and Z: 15.9776 Å. The present tool predicts docking result by obtaining energy value for each ligand. Finally, all the 27 ligands were analysed to detect binding position and energy value. The resultant structural complexes of the individual ligand/receptor binding were finally observed in AutoDoc tool,15 to determine some specific contacts between the atoms of the test compounds (ligands) and amino acids of the glycogen phosphorylase protein (receptor).

RESULTS AND DISCUSSION

Present *in silico* approach as molecular docking indicates that favourable binding energy value was observed. The

highest binding energy value was observed in Bicyclogermacrene (-6.4 Kcal/mol), followed by trans-Caryophyllene (-6.1 Kcal/mol), p-Cymene and Terpinolene (-6.0 Kcal/mol), Y-Terpinene, (E)-Tagetone (trans-tagetone) and (Z)-Ocimenone (cis-ocimenone) (-5.8 Kcal/mol) while lowest value was obtained in Octanal (-4.4 Kcal/mol) among other secondary metabolites of *T. errecta* in comparison with an established synthetic fungicide known Carbendazim (-6.7 Kcal/mol) and 2-Amino-2-hydroxymethyl-propane-1,3-diol (-3.5 Kcal/mol), a known inhibitory molecule (Table 2).

Different contact residues for each ligand were tabulated in Table 2. There was not found any hydrogen bonding for each test compound except 2-Methyl-1-butyl acetate showed one hydrogen bonding with ASN253 contact residue. It was observed in the docking pose and interaction, the close contact residues were found mainly ASN253, ASN255, THR196 and TRP374 for Bicyclogermacrene while ASN253, LEU195, THR196 and TRP374 for trans-Caryophyllene, ASN253, TYR337 and TRP374 for p-Cymene and Terpinolene, THR196, ASN253, TYR337 and TRP374 for y-Terpinene, (E)-Tagetone (trans-tagetone) and Ocimenone (cis-ocimenone) respectively. Only two contact residues such as TRP374 and ASN253 were obtained for Carbendazim and TRP374 for 2-Amino-2-hydroxymethylpropane-1,3-diol along with two hydrogen bonds connected amino acid residues such as ASN253 and GLU 193 (Fig 4-9A&B and Table 2).

Table 1: Established phytochemicals as volatile oil of *T. errecta* and synthetic fungicide

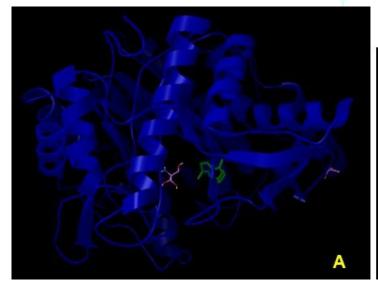
Sl. No.	Ligands	CAS no.*	Canonical SMILES*		
Phytoligands					
1.	Ethyl-2-methyl butanoate	7452-79-1	CCC(C)C(=0)OCC		
2.	2-Methyl-1-butyl acetate	624-41-9	CCC(C)COC(=0)C		
3.	α-Pinene	80-56-8	CC1=CCC2CC1C2(C)C		
4.	Camphene	79-92-5	CC1(C2CCC(C2)C1=C)C		
5.	Sabinene	3387-41-5	CC(C)C12CCC(=C)C1C2		
6.	β-Pinene	127-91-3	CC1(C2CCC(=C)C1C2)C		
7.	Myrcene	123-35-3	CC(=CCCC(=C)C=C)C		
8.	Octanal	124-13-0	CCCCCCC=O		
9.	α-Phellandrene	4221-98-1	CC1=CCC(C=C1)C(C)C		
10.	α-Terpinene	99-86-5	CC1=CC=C(CC1)C(C)C		
11.	p-Cymene	99-87-6	CC1=CC=C(C=C1)C(C)C		
12.	Limonene	138-86-3	CC1=CCC(CC1)C(=C)C		
13.	(Z)-β-Ocimene	3338-55-4	CC(=CCC=C(C)C=C)C		
14.	(E)-β-Ocimene	3779-61-1	CC(=CCC=C(C)C=C)C		
15.	Dihydrotagetone	1879-00-1	CC(C)CC(=0)CC(C)C=C		
16.	γ-Terpinene	99-85-4	CC1=CCC(=CC1)C(C)C		
17.	Terpinolene	586-62-9	CC1=CCC(=C(C)C)CC1		
18.	Linalool	78-70-6	CC(=CCCC(C)(C=C)0)C		
19.	allo-Ocimene	7216-56-0	CC=C(C)C=CC=C(C)C		
20.	(Z)-β-Ocimene oxide		CC(=CCC1C(O1)(C)C=C)C		
21.	(E)-Tagetone (trans-tagetone)		CC(=CC(=0)C=C(C)C=C)C		
22.	(Z)-Tagetone (cis-tagetone)	3588-18-9	CC(C)CC(=0)C=C(C)C=C		
23.	(Z)-Ocimenone (cis-ocimenone)		CC(=CC(=0)C=C(C)C=C)C		
24.	trans-Caryophyllene	87-44-5	CC1=CCCC(=C)C2CC(C2CC1)(C)C		
25.	Bicyclogermacrene		CC1=CCCC(=CC2C(C2(C)C)CC1)C		
Synthetic ligand Synthetic ligand					
1.	Carbendazim	10605-21-7	COC(=0)NC1=NC2=CC=CC=C2N1		
2.	2-Amino-2-hydroxymethyl-propane-1,3-diol	77-86-1	C(C(CO)(CO)[NH3+])O		

*Obtained from PubChem database

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Table 2: Selected phytochemicals of $\it T. errecta$ Linn. and synthetic compounds binding energy value after docking against β -glucosidase protein

Sl. No.	Ligands	Binding energy	Hydrogen bond no. and contact	Contact residues			
NO.		(Kcal/mol)	no. and contact				
Phyto	Phytoligands						
1.	Bicyclogermacrene	-6.4		ASN253, ASN255, THR196 and TRP374			
2.	trans-Caryophyllene	-6.1		ASN253, LEU195, THR196 and TRP374			
3.	p-Cymene	-6.0		ASN253, TYR337 and TRP374			
4.	Terpinolene	-6.0		ASN253, TYR337 and TRP374			
5.	γ-Terpinene	-5.8		THR196, ASN253, TYR337 and TRP374			
6.	€-Tagetone (trans-tagetone)	-5.8		THR196, ASN253, TYR337 and TRP374			
7.	(Z)-Ocimenone (cis-ocimenone)	-5.8		THR196, ASN253, TYR337 and TRP374			
8.	α-Terpinene	-5.7		ASN253, TYR337 and TRP374			
9.	Limonene	-5.4		ASN253, ASN255, TYR337 and TRP374			
10.	allo-Ocimene	-5.4		ASN255, TYR337 and TRP374			
11.	(Z)-Tagetone (cis-tagetone)	-5.3		THR196, ASN253, ASN255, TYR337 and TRP374			
12.	€-β-0cimene	-5.2		ASN253, TYR337 and TRP374			
13.	(Z)-β-Ocimene	-5.1		THR196, ASN253, TRP374 and TYR337			
14.	(Z)-β-Ocimene oxide	-5.1		THR196, TRP374, ASN253 and TYR337			
15.	α-Phellandrene	-5.0		TYR337, ASN253, ASN255 and TRP374			
16.	Sabinene	-5.0		TYR324, ARG231, GLU230 and GLU321			
17.	Linalool	-5.0		TYR337, ASN253, ASN255 and TRP374			
18.	β-Pinene	-4.9		ARG267, CYS270, PRO259 and TRP256			
19.	Myrcene	-4.9	5 1 1 X 775 (C)	TYR337, THR196, ASN255, ASN253 and TRP374			
20.	α-Pinene	-4.9	- 11 1 <u>- 2</u> 1 1 7 (TYR337, THR196, ASN255, ASN253 and TRP374			
21.	Dihydrotagetone	-4.7		ASN253, ASN255 and TRP374			
22.	Camphene	-4.7		GLU321, GLU320 and TYR324			
23.	2-Methyl-1-butyl acetate	-4.6	1 and ASN253	ASN255, LEU195 and TYR273			
24.	Ethyl-2-methyl butanoate	-4.5		TYR337, ASN253 and TRP374			
25.	Octanal	-4.4		TYR337, ASN253 and TRP374			
Synth	Synthetic ligand						
1.	Carbendazim	-6.7		TRP374 and ASN253			
2.	2-Amino-2-hydroxymethyl-	-3.5	2 and ASN253	TRP374			
	propane-1,3-diol		and GLU193				



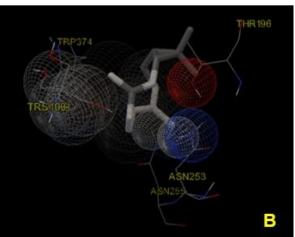


Figure 4: Bicyclogermacrene docking pose (A) and interaction (B)

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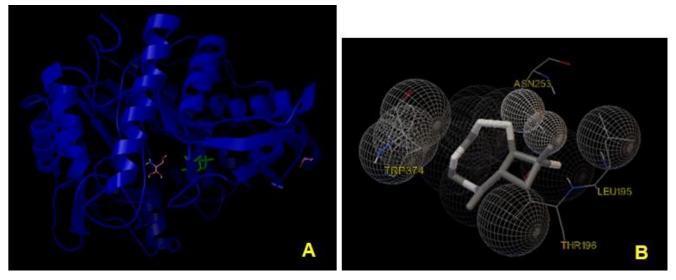


Figure 5: trans-Caryophyllene docking pose (A) and interaction (B)

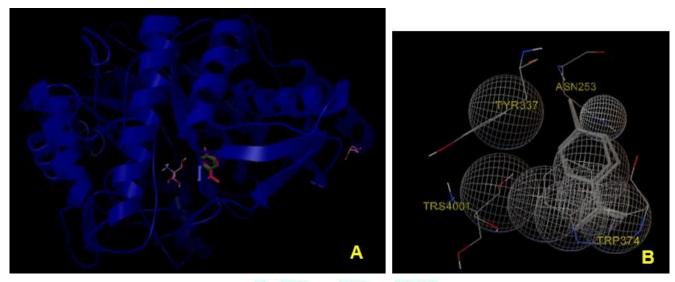


Figure 6: p-Cymene and Terpinolene docking pose (A) and interaction (B)

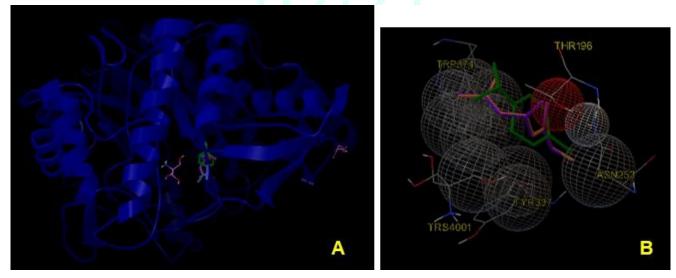


Figure 7: Y-Terpinene, (E)-Tagetone and (Z)-Ocimenone docking pose (A) and interaction (B)

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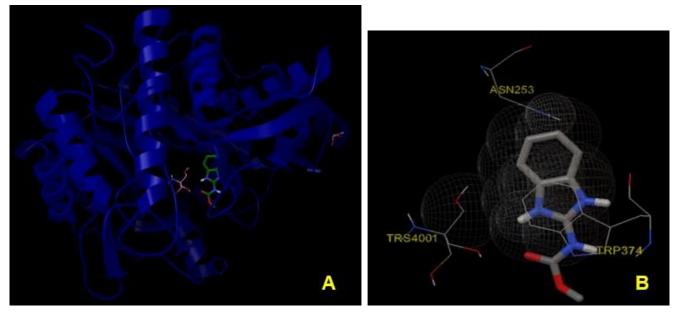


Figure 8: Carbendazim docking pose (A) and interaction (B)

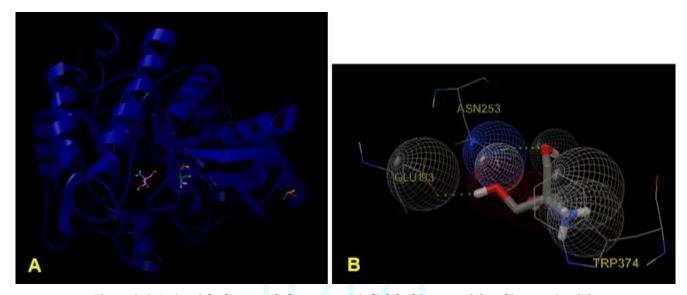


Figure 9: 2-Amino-2-hydroxymethyl-propane-1,3-diol docking pose (A) and interaction (B)

In the present computational screening, among established 25 phytoligands, suitable binding energy value was observed in Bicyclogermacrene, followed by trans-Caryophyllene, p-Cymene, Terpinolene Y-Terpinene, (E)-Tagetone (transtagetone) and (Z)-Ocimenone (cis-ocimenone) along with established synthetic fungicide Carbendazim against the βglucosidase receptor without any hydrogen bonding. Other 18 ligands were obtained below energy value than these ligands. The leaf phytocompounds of ornamental plant Tagetes errecta has potent antifungal effect on different fungal species.3 Moreover, natural compounds of plants can be suitable to prevent fungal resistance¹³ (Srivastava and Raveesha, 2016) because synthetic fungicide has showed toxicity and resistance to fungal species. 12 Interestingly, the alkaloid fraction of leaves of Prosopis juliflora have observed antifungal properties in experimental study while phytoligands such as Juliprosopine and Prosopine obtained potential to inhibit β -glucosidase by molecular docking.¹³

On the other hand, different β -glucosidases showed catalytic activity within or outside of the active site for enzymatic activity, which is still unclear to the researcher.²⁰ In the

present computational prediction highest binding energy was obtained for Bicyclogermacrene volatile oil found in leaves of T. errecta. It is well-known that inhibition of β -glucosidase activity of fungus prevent fungal pathogenicity in host plants. $^{13,21-23}$ Several experimental studies revealed that leaf volatile oil of T. errecta has potent antifungal activities, 5,24 but the prediction of lead compound(s) is an important task to know the design of fungistatic compound. Although, the isolation of lead compound(s) followed by in vitro and in vivo study is suggesting in future research.

CONCLUSION

In conclusion, prevention of fungal resistance onto crop species suitable lead molecule(s) of *T. errecta* can be used as fungistatic compound. However, it is suggesting further *in vitro* and *in vivo* assay with this phytoligand(s) after isolation from leaves to detect the inhibitory activity of different fungal strains and also toxicity evaluation to validate the present predictions.

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CONFLICT OF INTEREST: None

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