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In silico Study for Checking the Antifungal Properties of *Nyctanthes arbor-tristis*

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Abstract—*Nyctanthes arbor-tristis L., a large woody shrub admired for its fragrant white flowers is not only of great cultural importance but also finds immense use in indigenous medicine. Each and every part of the plant harbors a wide variety of active compounds and possesses antifungal, antibacterial, antiviral, laxative and diuretic properties amongst others. Leaf extracts of Nyctanthes were also found to be effective in inhibiting the radial growth of three fungal pathogens of rice viz. Pyricularia oryzae, Cochliobolus miyabeanus and Rhizotonia solani. The present in-silico study is an attempt to check the potential of antifungal activity of phytochemical constituents from Nyctanthes. 16 established phytoligands from seeds, leaves and flowers were docked against four enzymes involved in fungal infection – two cellulases (β-glucosidase and endo-β-1,4-glucan cellobiohydrolase) and two hydrolases (endopolygalacturonase and rhamnogalacturonase). Enzyme ligand binding energy was computed using PyRx software (Version 0.8) and the interactions were visualized in PyMol (Version 2.3). The binding energy of phytocompounds against cellulases came out to be comparatively higher than the hydrolases and the active compounds Arbortristoside D (-10.7 kcal/mol) and ursolic acid (-9.2 kcal/mol) showed greater affinities with them respectively. The result of this study highlights the potential of Nyctanthes arbor-tritis as an efficient deterrent for fungal growth and a preferable alternative for synthetic fungicide. Furthermore it suggests the need for wet lab research experiments to reaffirm these findings and to devise a method for the synthesis of selected active compounds at a commercial level.*

Introduction

The Sanskrit phrase "Paarinaha Samudrath jaatho va parijatah" means 'The Parijata tree emerged from the ocean after a profound search and is hence named so' [1]. *Nyctanthes arbor-tristis* (Parijata tree) Linn. is one of the most useful traditional medicinal plants in India. It is distributed widely in sub-Himalayan regions and Southwards to Godavari. Each part of the plant has some medicinal value and is thus commercially exploitable. It is now considered as a valuable source of several unique products for the medicines against various diseases and also for the development of some industrial products.This sacred tree with grey bark and milk-white fragrant flowers possesses antifungal, antibacterial, antiviral, antimalarial, anticancer, laxative and diuretic properties amongst others [2, 3]. Also called the night jasmine, this tree blooms at night and showers its flowers at the break of dawn [4]. Following the pattern of its flowering, the local people of Tripura are also known to predict weather and plan their agro forestry activities accordingly [5].

Each and every part of the plant harbors a wide variety of active compounds and is of immense importance in pharmacology. Leaf, flower, fruit and seed extracts of the plant are also reported to work against Gram negative bacteria [3]. Specifically, leaf extracts of *Nyctanthes* were also found to be effective in inhibiting the radial growth of three fungal pathogens of rice viz. *Pyricularia oryzae*, *Cochliobolus miyabeanus* and *Rhizotonia solani* [3]. The present *in silico* study is aimed to check the potential of antifungal properties of phytochemical constituents from *Nyctanthes arbor-tristis*. The approach of this study is based on molecular docking to determine the interactions between the selected phytoligands and certain fungal enzymes- two cellulases and two hydrolases. Molecular docking softwares utilize various algorithms for predicting all the possible interactions between ligands and the macromolecule and also estimate their binding energy [6, 7]. The fungal enzymes considered as target proteins in the study are, endopolygalacturonase and rhamnogalacturonase (hydrolases) which are involved in initial stages of cell wall degradation and β-glucosidase and endo-β-1,4-glucan cellobiohydrolase (cellulases) which come into function after appressorium formation [8]. The fungal enzymes were also docked against their respective inhibitors as a reference point for evaluating and comparing the strength of binding energy of all the phytoligands with them. Endo-β-1,4-glucan cellobiohydrolase shows product inhibition and thus was docked against cellobiose [9], β-glucosidase with gluconolactone [10] and

endopolygalacturonase and rhamnogalacturonase with epicatechin, a phenolic compound which is an inhibitor of hydrolases as a part of plant's natural defense system [8].

2. Methodology

2.1. Selection of fungal enzymes

The crystal protein structure of two cellulases- β- glucosidase [11] and endo-β-1,4-glucan cellobiohydrolase [12] and two hydrolases – Endopolygalcturonase [13] and Rhamnogalacturonase [14] were retrieved from Protein Data Bank (https://www.rcsb.org/). Table 1 lists the fungal enzymes taken into consideration with their PDB ID and authors and Figure 1 depicts their three dimensional structure. These structures are color coded according to their secondary structures, where pink color represents alpha helix, yellow for beta pleated sheets and blue for loops.

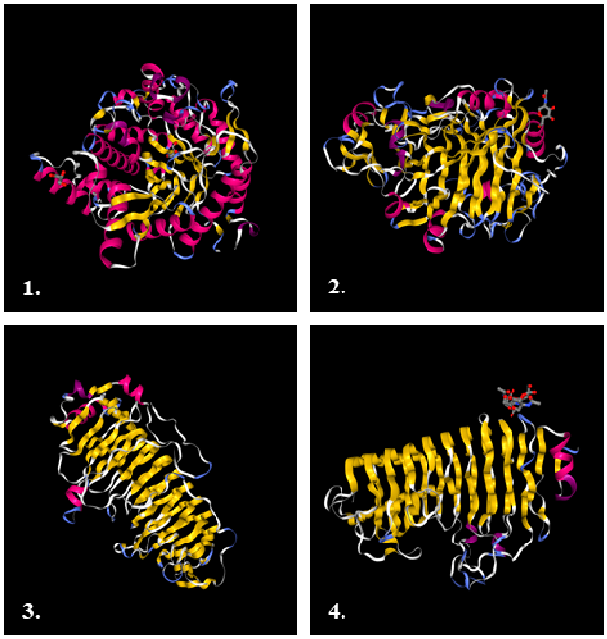

Figure 1: Three dimensional structure of 1. β-glucosidase, 2. Endo-β-1,4-glucan cellobiohydrolase, 3. Endopolygalacturonase and 4. Rhamnogalacturonase (structures retrieved from PDB)

Table 1. Selected fungal enzymes

Target protein (fungal enzyme)	PDB ID	Author
β-glucosidase	3AHZ	Jeng *et al.*, 2010
Endo-β-1,4-glucan cellobiohydrolase	1GPI	Munoz, *et al.*, 2002
Endopolygalcturonase	1HG8	Federici, *et al.*, 2001
Rhamnogalacturonase	1RMG	Petersen *et al.*, 1998

2.2. Selection of phytoligands

The phytochemical constituents of *Nyctanthes arbor-tristis* were selected by studying literature and previous works done on the plant [2, 3, 15, 16]. The Canonical SMILES (Simplified Molecular-Input Line-Entry System) of 16 established phytoligands from seeds, leaves and flowers were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [17]. The SMILES were then converted into 3D structures and were saved as PyRx (Version 0.8) software compatible.pdb files from CORINA online server (https://www.mn-am.com/online_demos/corina_demo) [18]. Table 2 lists the selected phytoligands along with their source and Figure 2 depicts their chemical structure.

2.3. Molecular docking and visualization of interaction

Each enzyme was docked against all the phytoligands and binding energy for each of them was computed using autodock vina wizard of PyRx (Version 0.8) software [19]. The docking grid on the target protein was (by default of) the dimensions X: 25 Y: 25 Z: 25. The output files were saved in .pdbqt format and the enzyme ligand interactions were then visualized in PyMol (Version 2.3) [20] for observing any polar contacts between them. All the fungal enzymes were also docked against their known inhibitors – β-glucosidase with gluconolactone, endo-β- 1,4-glucan cellobiohydrolase with cellobiose, endopolygalcturonase and rhamnogalacturonase with epicatechin as a reference point for evaluating and comparing the strength of binding energy of all the phytoligands with the fungal enzymes.

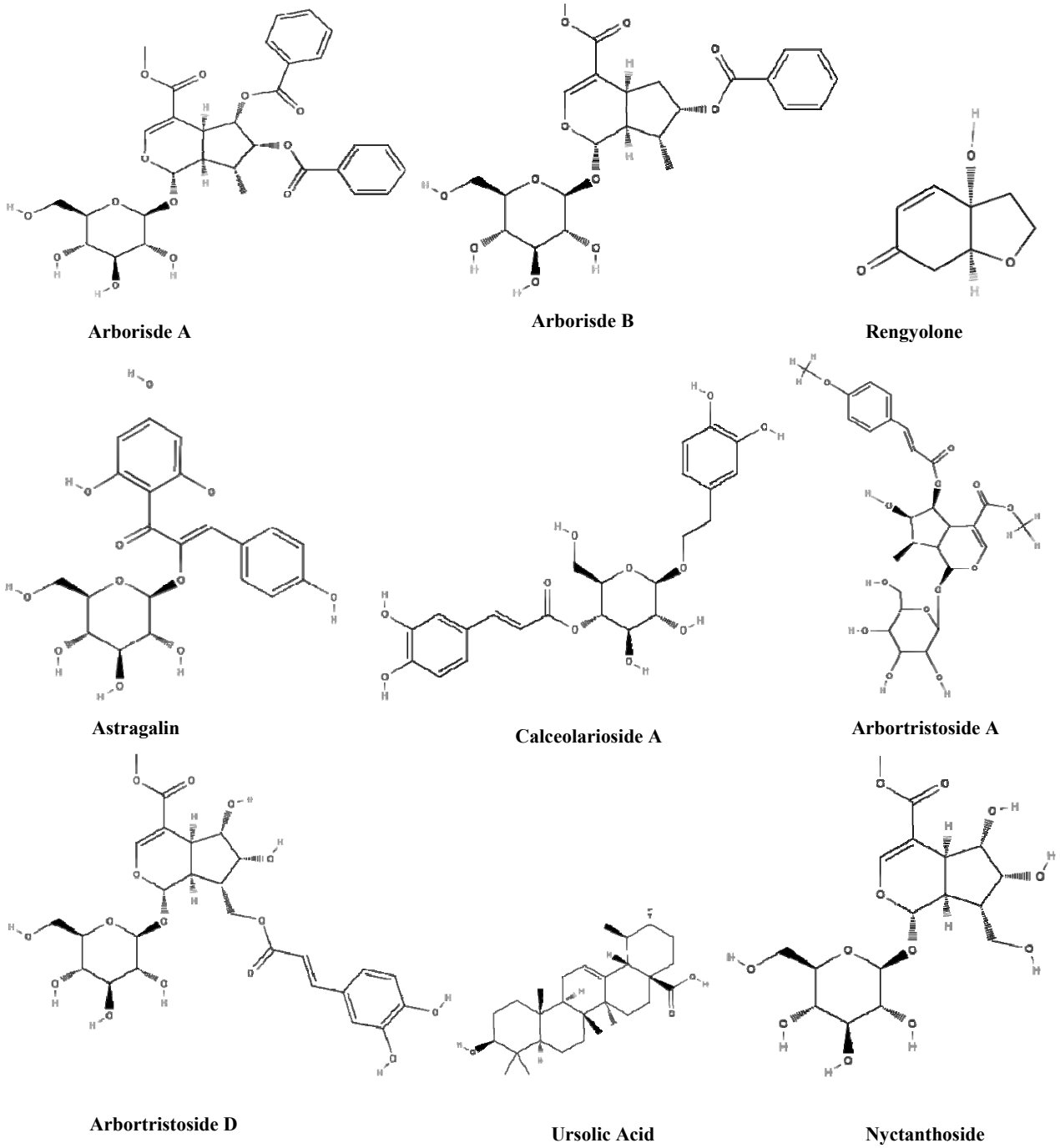

Figure 2: Chemical structures of active compounds of *Nyctanthes arbor-tristis*

Table 2: Selected phytoligands from *Nyctanthes arbor-tristis* m *Nyctanthes arbor-tristis.*

Source	Ligands	Canonical SMILES
Seeds	1. Arbortristoside A	CC1C2C(C(C1O)OC(=O)C=CC3=CC=C(C=C3)OC)C(=COC2OC4C(C(C(C(O4)CO)O)O)O)C(=O)OC
	2. Arbortristoside B	COC(=O)C1=COC(C2C1C(C(C2CO)O)OC(=O)C=CC3=CC(=C(C=C3)O)O)OC4C(C(C(C(O4)CO)O)O)O
	3. Arbortristoside C	CC1C2C(C(C1OC(=O)C=CC3=CC=C(C=C3)O)O)C(=COC2OC4C(C(C(C(O4)CO)O)O)O)C(=O)OC
	4. Arbortristoside D	COC(=O)C1=COC(C2C1C(C(C2COC(=O)C=CC3=CC(=C(C=C3)O)O)O)O)OC4C(C(C(C(O4)CO)O)O)O
	5. Arbortristoside E	CC1C2C(C(C1O)O)C(=COC2OC3C(C(C(C(O3)COC(=O)C=CC4=CC=C(C=C4)OC)O)O)O)C(=O)OC
	6. 6-β-hydroxyloganin	CC1C2C(C(C1O)O)C(=COC2OC3C(C(C(C(O3)CO)O)O)O)C(=O)OC
Leaves	7. Arborside A	CC1C2C(C(C1OC(=O)C3=CC=CC=C3)OC(=O)C4=CC=CC=C4)C(=COC2OC5C(C(C(C(O5)CO)O)O)O)C(=O)OC
	8. Arborside B	CC1C(CC2C1C(OC=C2C(=O)OC)OC3C(C(C(C(O3)CO)O)O)O)OC(=O)C4=CC=CC=C4
	9. Arborside C	CC1C2C(C(C1OC(=O)C3=CC=CC=C3)O)C(=COC2OC4C(C(C(C(O4)CO)O)O)O)C(=O)OC
	10. Arborside D	COC(=O)C1=COC(C2C1C(C(C2COC(=O)C3=CC=CC=C3)O)O)OC4C(C(C(C(O4)CO)O)O)O
	11. β-sitosterol	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
	12. Calceolarioside A	C1=CC(=C(C=C1CCOC2C(C(C(C(O2)CO)OC(=O)C=CC3=CC(=C(C=C3)O)O)O)O)O)O
	13. Ursolic Acid	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1C)C)C(=O)O
Flowers	14. Nyctanthoside	COC(=O)C1=COC(C2C1C(C(C2CO)O)O)OC3C(C(C(C(O3)CO)O)O)O)O
	15. Rengyolone	C1COC2C1(C=CC(=O)C2)O
	16. Astragalin	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)O)O

Results and Discussion

Results of this study showed favorable binding energy for all the phytoligands with the target proteins except the binding energy of Arbortristoside B with rhamnogalacturonase enzyme which turned out to be + 5.8 kcal/mol. The docking of Arbortristoside D with endo-β-1,4-glucan cellobiohydrolase yielded the highest binding energy with a value of -10.7 kcal/mol followed by Arbortristoside E and Calceolarioside A with a binding energy of -10.4 kcal/mol. While studying (Table 3) the binding energy of all the phytoligands with the selected four fungal enzymes it was observed that the ligands showed higher binding energy (average) with cellulases (-8.631±1.23 kcal/mol) than hydrolases (-6.123±2.53 kcal/mol) .

The ligands– Arbortristoside D, Astragalin, Ursolic acid and Calceolarioside A showed highest binding energies with endo-β-1,4-glucan cellobiohydrolase, β-glucosidase, endopolygalacturonase and rhamnogalacturonase respectively and were selected for visualization of interaction in PyMol (Version 2.3). The

Table 3: Binding energy of phytoligands from *Nyctanthes arbor-tristis* with the fungal enzymes

Sno.	Ligands	Binding energy of ligands with individual enzymes (kcal/mol)				Average binding energy of ligands (kcal/mol)
		β- glucosidase	Endo β -1,4-glucan cellobiohydro-lase	Endopolyga-lcturonase	Rhamnogalac-turonase	
1.	Arbortristoside A	-7.8	-9.2	-7.7	-5.6	-7.575±1.48
2.	Arbortristoside B	-8.7	-7.5	-4.2	+5.8	-3.65±6.58
3.	Arbortristoside C	-7.7	-9.6	-8.7	-5.6	-7.9±1.71
4.	Arbortristoside D	-7.9	**-10.7**	-7.8	-6.3	-8.175±1.83
5.	Arbortristoside E	-8.2	-10.4	-7.1	-6.5	-8.05±1.71
6.	Ursolic acid	-8.0	-10.0	**-9.2**	-4.2	-7.85±2.56
7.	Nyctanthoside	-7.1	-8.6	-6.9	-5.3	-6.975±1.35
8.	Rengyolone	-5.6	-6.2	-5.4	-4.6	-5.45±0.66
9.	β-sitosterol	-7.5	-9.3	-6.8	-5.4	-7.25±1.62
10.	Astragalin	**-9.3**	-9.7	-7.9	-6.3	-8.3±1.54
11.	Arborside D	-7.9	-9.5	-7.5	-5.8	-7.675±1.51
12.	6-β-hydroxy-loganin	-7.3	-8.3	-7.6	-5.0	-7.05±1.42
13.	Calceolarioside A	-8.7	-10.4	-8.0	**-7.3**	**-8.6±1.32**
14.	Arborside A	-8.8	-10.0	-8.1	-5.4	-8.075±1.94
15.	Arborside B	-9.1	-8.9	-7.4	-5.9	-7.825±1.49
16.	Arborside C	-8.0	-10.3	-7.7	-6.1	-8.025±1.73

ligand Arbortristoside D formed 12 polar contacts with Arg 104, Tyr 168, Asp 170, Gln 172, Glu 212, Arg 240, Asp 366 and Arg 391 residues of endo-β-1,4-glucan cellobiohydrolase; Astragalin formed 5 polar contacts with Asn 253, Asn 255, Tyr 273 and Glu 451 residues of β-glucosidase; Ursolic acid formed 1 polar contact with His 128 residue of endopolygalacturonase and Calceolarioside A formed 12 polar contacts with Arg 128, Arg 131, Asp 197, Lys 203, Ser 226, Asn 247, Gln 248 and Lys 253 residues of rhamnogalacturonase.

The binding energy of inhibitors with the fungal enzymes, β-glucosidase was -5.2 kcal/mol, rhamnogalcturonase was -5.7 kcal/mol, endo-β- 1,4-glucan cellobiohydrolase was -7.1 kcal/mol and endopolygalacturonase was -7.5 kcal/mol. These binding energies were more or less equivalent to the binding energies of phytoligands with the fungal enzymes, indicating that the phytoligands under study are capable of inhibiting the enzymes by binding to their pocket.

1.

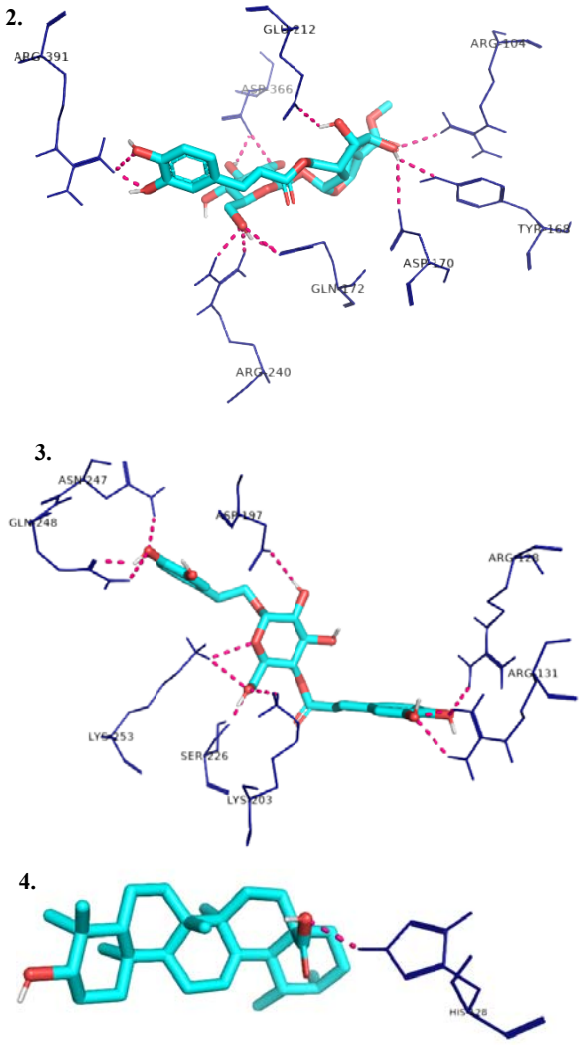

Figure 5: Interaction of 1. β-glucosidase with Astragalin 2. Endo-β-1,4-glucan cellobiohydrolase with Arbortristoside D 3. Rhamnogalacturonase with Calceolarioside A and 4. Endopolygalacturonase with Ursolic acid visualized by PyMol (Version 2.3)

Cellulases and Hydrolases are attractive targets for preventing fungal infections [21]. Previous *in silico* study, supported by wet lab experiments has demonstrated the role of Juliprosopine and Prosopine from *Prosopis juliflora* in inhibiting β-glucosidase activity [21]. Plants are also known to inhibit infection-initiating enzymes through various natural defense molecules such as PGIPs, which are involved in inhibiting the fungal polygalacturonase [22]. Fungal hydrolases are inhibited by phenolic compounds known as phytoalexins, epicatechin being one of them and used as a reference point in the present study [8]. *In silico* approaches for checking antifungal action, such as that of Lahiri et. al. on the plant *Tagetes erecta* [18], although require validation from *in vitro* experimentation but are very useful in narrowing down the candidates

Conclusion

The active compounds of *Nyctanthes arbor-tristis* were able to bind to the pocket of cellulases and hydrolases *in silico*. Inhibition of fungal enzymes is also observed as a part of plant's natural defense mechanism, which suggests that the fungal enzymes could be used as potent targets for prevention of fungal infection. The computed binding energies of the selected phytoligands from *Nyctanthes* were comparable to the binding energy of the fungal enzymes with their known inhibitors. Some of the phytoligands (Calceolarioside A, Astragalin, Arbortistoside D, Arobortristoside E, Arborside A) even scored higher than the inhibitors selected for reference in the study.

This study clearly reflects the ability of *Nyctanthes* as an alternative to synthetic fungicides, which are susceptible to resistance development by fungal pathogens. These results can further be investigated by *in vivo* experiments. In future a commercially viable method to synthesize these active compounds could be developed.

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