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Docking studies of andrographolide and its *In vitro* validation

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Abstract
In silico molecular docking is one of the most popular molecular modeling approach, used to predict binding poses of ligands to target proteins (TP). It can be used to predict the affinity of ligands to TP. Andrographolide, a biomolecule of plant *Andrographis paniculata*, with medicinal property will be studied for finding interaction with TP (diseased proteins of human and plant origin). Nine different target proteins HIV protease, THNR reductase, trichothecene-3-O-acetyl transferase, cyclooxygenase, acetylcholine esterase, polygalacturonase, glucosamine-6-phosphate synthase, phospho- shikimate transferase and N-acetyl-β-D-hexosaminidase will be docked with andrographolide using Argus 4.0 bioinformatics software. After successful docking *in vitro* validation of andrographolide activity will be checked by subjecting *S.rolfsii, R.solani* and *F.udum* fungus disease causing agents of tomato with Andrographis extract and efficacy will be evaluated. These results anticipate the application of andrographolide as a bioagent against bacteria, fungi, virus, etc, and will prove its application in agriculture also, this bioactive compound, andrographolide can serve as a potent source of pesticide /insecticide of biological origin. This will give a way for the formulation of new biopesticide which can serve as a better alternative to chemical pesticides.

Keywords: TP, HIV protease, molecular docking, andrographolide, biopesticides, etc

Introduction
Andrographolide (AG) is the most potent labdane diterpenoid produced in *Andrographis paniculata* herb which has several pharmacological properties responsible for antibacterial, antifungal, hepatoprotective and anti-diabetic property. Its pure form 98 % costs Rs. 4106.59/100 mg. Its biopesticidal properties can be utilized against plant pest and pathogens due to need of organic products and agriculture produce free from chemicals residues. This is the reason for studying andrographolide for finding interaction with target proteins. Molecular docking is a key tool in structural molecular biology to predict binding poses of ligands to target proteins. This technique enables us to find out the interaction between a specific compound and target protein which will help us to elucidate function of the compound. Nine different target proteins (four human and five plant disease proteins)will be used in this research work which included HIV protease (1D4Y: TP causing HIV in human), THNR reductase (1YBV: TP causing rice blast disease), trichothecene-3-O-acetyl trans ferase (2RKV: TP causing Fusarium head blight in plants), cyclooxygenase (1PXX: TP causing inflammation and pain in human), acetylcholine esterase (2ZJU: TP causing nerve impulse transmission in human), polygalacturonase (1HG8: TP causing Fusarium wilt in plants), glucosamine-6-phosphate synthase (1JXA: TP causing fungal infection in human), phospho-shikimate transferase (3TR1: TP causing function in herbs, fungi etc) and N-acetyl-β-D-hexosaminidase (3NSN: TP causing function in insects and microorganisms). These target proteins were selected on the basis of previous studies on andrographolide interaction with human proteins and for its utilization in agriculture we selected other six target proteins. This will give a preliminary idea for activity of andrographolide against plant pathogens which will also be validated *in vitro* using major plant pathogens *S.rolfsii, R.solani* and *F.udum.* The research will be conducted with two different objectives as discussed below:-

1. *In silico* molecular docking studies on *A. paniculata*
In silico molecular docking, is one of the most popular molecular modelling approach that is used to perform simulations for predicting binding poses of ligands to proteins (Zoete *et al*., 2009). This offers elevated screening capacity and supports to formulate simple, clear needs to

candidate drugs, which allows implementation of rational drug design (Klaus *et al.*, 2001). The potential of andrographolide using *In silico* docking analysis will be checked towards various diseased proteins of plant and animal origin.

a) Andrographolide (Ligand), (Pub Chem CID 5318517 and ZINCID ZINC03881797)

Andrographolide is a major active compound of *A. paniculata*, which is a labdane diterpenoid. Andrographolide (Fig. 1, Table 1) has an alkylidene-γ- butyrolactone, two olefins bonds at C-8 and C-12 and three hydroxyl at C-3, C-19 and C-14 (Nanduri *et al.*, 2004) [3]. The structure of andrographolide has been analyzed by X-ray crystallographic method and the proposed systemic name is 3-[2-decahydro-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-2- methylene-1-naphthalenyl1] ethylidine] dihydro-4-hydroxy-2(3H)-furanone (Smith *et al.*, 1982).

Based on computational study using Auto Dock and MOE softwares andrographolide is assigned as a flexible ligand which has eleven conformers and six rotational bond (Levita *et al.*, 2009) [1]. This flexibility allows andrographolide to be able to interact with different targets.

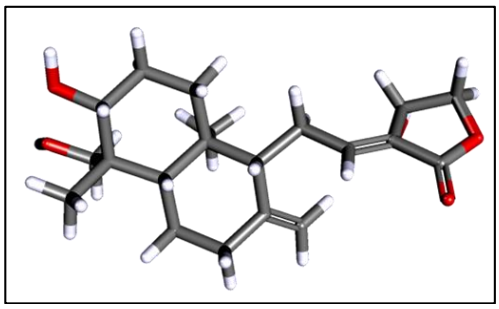

Fig 1: 3D andrographolide structure

Table 1: Physical representations for andrographolide

pH range	Xlog P	Apolar Desolvation (kcal/mol)	Polar Desolvation (kcal/mol)	H-bond donars	H-bond acceptors	Net charge	Molecular Weight (g/mol)	Rotatioal bonds
Reference (pH)	2.9	2.57	-13.85	3	5	0	350-455	3

***Source*:** ZINC database

b) Target proteins (Receptor)

Nine proteins (Fig. 2) responsible for causing diseases in human and plants were docked with andrographolide. Purpose behind docking is easy inhibition. If andrographolide is docked to diseased human and plants it will inhibit the enzymatic activity thereby curbing spread of disease. List of protein that has been docked with andrographolide is presented below (Table 2):

Table 2: Target proteins, PDB ID, diseases/function and functional area

S. No	Name of target protein (Receptor)	PDB ID	Diseases / function	Functional area (human or plants)
1	HIV Protease	1D4Y	HIV	Human
2	THNR Reductase	1YBV	Rice blast disease	Plants
3	Trichothecene-3-O-acetyl transferase	2RKV	Fusarium head blight	Plants
4	Cyclooxygenase-2	1PXX	Inflammation and pain	Human
5	Acetylcholine esterase	2ZJU	Nerve impulse transmission	Human,insect,etc
6	Polygalcturonase	1HG8	Fusarium wilt	Plants
7	Glucosamine-6-phosphate synthase	1JXA	Antifungal	Human
8	Phosphoshikimate transferase	3TR1	Function in herbs, fungi etc	Plants
9	N-acetyl-β-D-hexosaminidase	1NSN	Function in insects and microorganisms	Plants

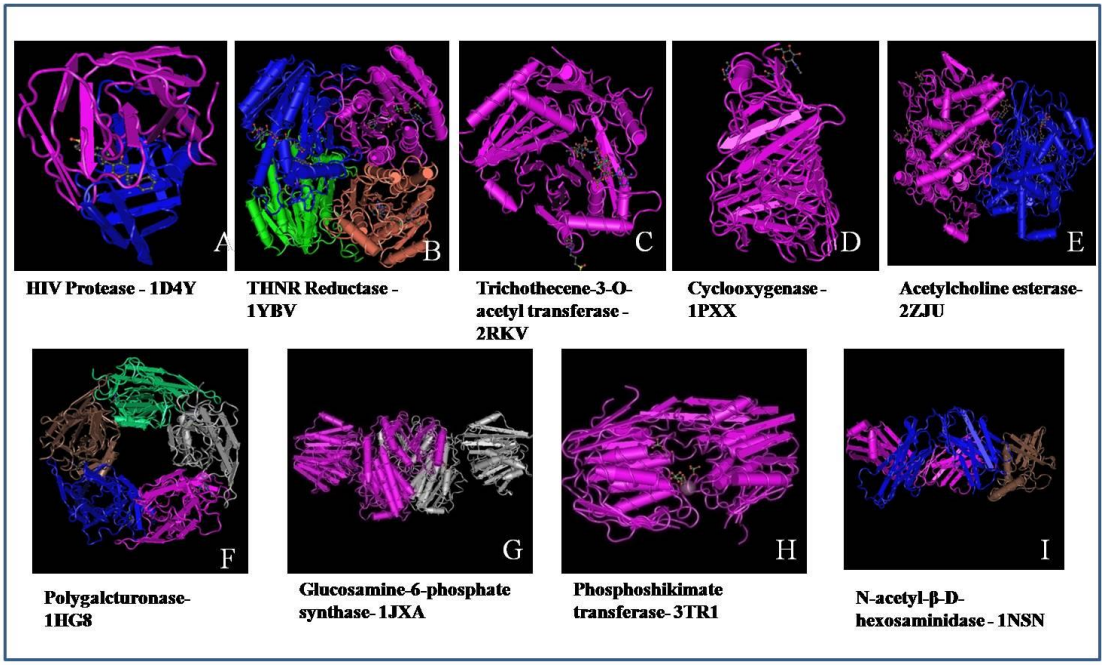

Fig 2: Ribbon structure of target proteins (Source:- Brookhaven protein data bank (*www.rcsb.org)*

c) Argus lab software for docking analysis

Argus lab 4.0 (Meshram and Jangle, 2009) [2] distributed freely for windows platforms by Planaria software, has become a favourite introductory molecular modelling package with academics mainly because of its user-friendly interface and intuitive calculation menus.

2. Bioefficacy of *A. paniculata* against major crop pathogen

a) Plant Material

Leaves of *A. paniculata* will be collected from the Department of Plant Molecular Biology and Biotechnology, I.G.K.V, Raipur and surface sterilized with 0.01 % $HgC1_2$ and then shade dried followed by drying in hot air oven below 60º C for 30 minutes. The dried plant leaves were then pulverized into coarse powder using mortar and pestle. The dried powder was then used for extraction with 80 % ethanol.

b) Fungal strains

The three test organisms *S. rolfsii*, *R. solani* and *F. udum* (Fig. 3.3) were used, supplied by the Department of Plant pathology, I.G.K.V, Raipur. The organisms were sub-cultured on potato dextrose agar media, incubated at 37°C for 4-6 days and stored at 4°C in the refrigerator to maintain stock culture.

c) Solvent extraction of powdered leaf material

The coarse dried powder of the leaves will be extracted using microwave followed by shaking in shaker for 1 hour and centrifuged at5000 rpm for 15 minutes. The ethanol extract obtained will be collected, filtered using filter membrane (0.45µ) and concentrated in vacuum under reduced pressure by suction pump and Andrographis powder will be recovered.

d) Inhibitory assay

Inhibitory effect of plant extract on three fungal pathogens was assessed using poisoned food technique (Y.L. Nene and P.N. Thapliyal, 1979). Different concentrations i.e. 1%, 2%, 3%, 4% of plant leaf extract will be prepared by adding required quantity of double autoclaved sterile water. 1 ml of each concentration will be aseptically poured into the petriplates followed by the addition of Potato dextrose agar medium (20 ml). After solidification of media, 5 mm disc from actively growing margins of test fungi colony obtained from 4-6 days old stock culture will be inoculated at the centre of media plate and incubated at 25±2°C. The colony diameter in each treatment was measured on the 2nd day and 6th day after inoculation. The diameter of inhibition zone will be measured and the percent inhibition will be calculated using formula:

$$\text{Inhibition percentage} = \frac{\text{Diameter of colony in control} - \text{Diameter of colony in treatment}}{\text{Diameter of colony in control}} \text{X } 100$$

The research work will help to get a preliminary idea of interaction of andrographolide with different target proteins hence for *in vitro* validation, time will be saved and only the successfully docked target proteins will be choosen for further validation. This approach will enable us to have a biomolecule which can be used against some important plant pathogens. This will encourage the use of andrographolide for organic farming which is an important issue now a days.

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