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RESEARCH ARTICLE

IN-SILICO INHIBITORS FOR PECTIN- METHYLESTERASE OF *A. THALIANA* BY VIRTUAL SCREENING TO INHIBIT THE SEED GERMINATION PROCESS

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ABSTRACT

Pectin is the one of the major component of plant cellwall which maintains structural integrity of cellwall. They can be modified by pectinases such as pectin methylesterases (PMEs) which catalyses the demethylesterification of homogalacturonans releasing pectate and methanol, playing important roles in growth, development and seed germination. The activity of an enzyme PME affects the cell wall porosity and elasticity by allowing water uptake. The control PME activities by several proteinaceous inhibitors have a direct effect on the regulation of various processes in plant physiology. Recently it has been found that the catechin isolated from Green tea can inhibit the PME from citrus and tomato plants. In the previous work we have characterized the PME from *A. thaliana* & predicted its 3D structure by using bioinformatics tools. Now, the present study focuses to find out the novel inhibitor of PME by screening the various catechin analogue compounds from the ZINC database. The molecular docking studies of catechin analogue were carried out by using the Hex software against the PME of *A. thaliana* which give the detailed insight of interacting residue with the ligand and target complex in the Pymol software.

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INTRODUCTION

The plant cell wall is composed of polysaccharides along with proteins & aromatic substances. The wall polysaccharides are often classified into cellulose, hemicelluloses, and pectin and these three types are represented in almost all cell walls in varying proportions (Jesper Harholt *et al*., 2010). Pectin is consists of four different homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan I (RG- I), rhamnogalacturonan II (RG- II) (Jesper Harholt *et al*., 2010). This is one of the principal of cellwall components involved in temporal and spatial regulation of the growth process. The pectin homogalacturonan composed of a linear chain of 1, 4-linked a-D-galacturonic acid (GalUA) residues, the homogalacturonans can be methylesterified at the C-6 carboxylic acid groups of the GalUA residues (Fry, 2000; Wolf *et al*., 2009). Through biochemical changes to pectin composition and biochemical configuration, the properties of this material can be altered to trigger specific developmental processes (Ridley *et al*., 2001). It has been indicated that enzyme pectinmethylesterases plays important role in seed germination process. During the process of seed germination the cell wall of the radical and of the tissues around it must expand (Robert Palin and Anja Geitmann, 2012). The pectin methylesterases (PMEs) (EC 3.1.1.11) remove some of the methyl ester groups from HG, releasing methanol and protons. Pectinmethylesterase (PME) catalyzes reactions according to the double-displacement mechanisms, de-esterification through transferring the C6 carboxyl groups in the pectin-PME complexes to water molecules altering the degree and pattern of methyl esterification and trans acylation through transferring the C6 carboxyl groups to the hydroxyl groups of another pectin molecules and resulting in the formation of high molecular weight pectins with new non-methoxy ester linkages which facilitate plant cell wall modification and subsequent breakdown allowing water uptake (Müller *et al*., 2013). PME activity thus alters cell walls and, hence, mediates various physiological and biochemical processes in plants, including elongation, growth, fruit ripening, seed germination (Jiang *et al*., 2001) and resistance to diseases (Micheli, 2001). The control of methylesterification levels by PMEs has been recently reported to have a direct effect on the regulation of a wide range of processes in plant physiology. One aspect of PME regulation is the inhibition by specific proteinaceous PME inhibitors (PMEIs). The degree of pectin methylesterification of the cell walls of seed tissues influences

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the rate of germination of the seeds. Several PMEIs from Arabidopsis (*Arabidopsis thaliana*) and other plant species have been characterized (Pooja Kohli *et al*., 2015; Wolf *et al*., 2003). However, the use of proteinaceous inhibitors is complex and hence not trivial. Small molecule inhibitors would be more tractable as applied enzyme inhibitors (Raiola *et al*., 2004). Recently Lewis *et al* identified the green tea catechin epigallocatechin gallate as a natural inhibitor of pectin methyl esterases by gel assay in tomato (*Solanum lycopersicum*) & citrus (Lewis *et al*., 2008). Green tea is a rich source of catechins, which account for up to 30% of the leaf dry weight (Graham, 1992). The catechins are polyphenolic structure of C6–C3–C6 with two aromatic rings and several hydroxyl groups. The catechins are classified into two groups; free catechins and esterified catechins. The free catechins are catechin, gallocatechin, epicatechin (EC), epigallocatechin (EGC), whereas the esterified catechins are EGCG, epicatechin gallate (ECG), gallocatechin gallate (GCG), and catechin gallate (CG) (Quan *et al*., 2010). Even though *A. thaliana* is a model plant, the physicochemical & structural details of its PME are not available. In the absence of this experimental data on the physicochemical & structural details of PME of *A. thaliana*, *In- silico* approach with its ability to predict its physicochemical & structural properties offers reasonable alternative. In the previous work we have characterized the PME of *A. thaliana* & predicted its 3D structure using bioinformatics tools. The PME of A. thaliana contains 595 amino acids & the physicochemical properties depict that the PME is alkaline, stable, cationic protein. The secondary structure reveals that PME consist of a helix, a sheet and random coil structure within its short stretch of residues. The 3D structure predicted by SWISS-MODEL was validated using PROCHECK, the percentage of most favorable region was 86.1% (Yogesh N Joshi *et al*., 2016). In continuing with the previous work the present study is focuses on the identification binding site in the PME of A. thaliana & screening of various catechin analogue compounds from the ZINC database (contains over 21 million compounds) using the HEX molecular docking software against the PME from the A. thaliana. Our screening approach identified nine molecules with ZINC ID & docking score Catechin (Id- zinc_4114953, Docking score=-233.20), 2-(3,4-Dihydroxyphenyl) chroman-3,5,7-triol (Id- zinc_ 397894, Docking score: -233.20), Epicatechin (Id- zinc_14436215, Docking score: -233.20), Epigallocatechin (Id- zinc_4214953, Docking score: -232.38), (2S,3R)-2-(3,4,5-Trihydroxyphenyl) chroman-3,5,7-triol/Gallocatechin (Id- zinc_3870337, Docking score: -238.56), Epicatechin gallate (Id- zinc_4534390, Docking score: -285.73), 3-Galloylcatechin (Id-zinc_ 6040160, Docking score:-281.83), Epigallocatechin gallate (Id-zinc_3870412, Docking score: -238.73), Gallocatechin gallate (Id- zinc_3470412, Docking score:-238.73). As the Epicatechin gallate & 3- Galloylcatechin can binds to the PME with high docking score, the further invitro studies of effect of inhibition of the PME by the catechin analogues provide the best alternative to stop the inhibition of the seed germination process of the weeds, unwanted plants & poisonous plants.

MATERIALS AND METHODS

1. Prediction, Validation & Visualization of 3D structure of PME

The 3D structure of PME of *A. thaliana* was predicted by using Swiss-model server (Marco Biasini *et al*., 2014; Arnold *et al*., 2006; Kiefer *et al*., 2009; Guex *et al*., 2009). The selection of template was accomplished by protein BLAST using PDB database having identity more than 30%. The evaluation and validation of generated model was performed with PROCHECK server on PDBSum database (Laskowski *et al*., 1993) and predicted model was visualized by Rasmol visualization tool (Sayle and Milner-White, 1995).

2. Active site prediction of PME

The active site of PME protein was predicted by using CASTp (Computed Atlas of Surface Topography of proteins) server (Sathish Kumar Paramashivam *et al*., 2015). The identification of active sites is often the starting point for protein function, annotation and molecular docking.

3. Designing the ligand Library using ZINC database

The natural inhibitor catechin (CID: 73106) for PME was downloaded from PubChem chemical database in Mol2 chemical file format. PubChem is a database of chemical molecules and their activities against biological assays (Laskowski *et al*., 2014). The analogues of catechin molecule were generated by using ZINC database. ZINC, a free database for virtual screening' contains over 21 million compounds in ready-to-dock, 3D formats, available at the URL http://zinc.docking.org (Irwin and Shoichet, 2005). The analogues were analyzed by MarvinView chemical viewer package. It allows for analysing easy scrolling of thousand of molecules either in a grid view or in a spreadsheet view. The analogues were saved in PDB format by using the Mravinsketch (McBride, Ryan, 2012).

4. Toxicity prediction

The ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of the target chemical compounds were calculated using admetSAR which describes the deposition of chemical compounds within an organism. Blood-Brain Barrier (BBB) penetration, HIA (Human Intestinal Absorption), and AMES toxicity were calculated. The cytochrome P450 super family plays an important role in drug metabolism and clearance in the liver (Balani *et al*., 2005).

5. Molecular Docking

The molecular docking analysis of between catechin and its analogue with enzyme PME was carried by Hex 8.0 molecular docking software. Hex is an interactive protein docking and molecular superposition program. Hex package reads protein structures in PDB format and small-molecules in SDF files (Macindoe *et al*., 2010). It is an interactive molecular program for calculating and displaying feasible acids and small biomolecules with binding or docking score. The further docked complex was visualized by PyMOL visualizations tool for interaction studies.

RESULTS AND DISCUSSION

1. Prediction, Validation & Visualization of 3Dimensional structure of PME

The homology modeling of PME from *A. thaliana* was obtained through SWISS MODEL server using resolution 1.8

A° structure of PME from *Daucus carota* (PDB Id:1gq8.1 Chain A) as a template. The evaluation and validation of generated model were executed with PROCHECK server on PDBSum database which is shown in Fig. I. Validation of the predicted PME from *A. thaliana* by PROCHECK analysis showed that 86.1% of the residues of PL model were present in the most favoured region followed by 13.9% in the allowed region, 0% in generously allowed region and disallowed region respectively of Ramachandran plot which are shown in Table I. Further the predicted structure was visualized by Rasmol viewer which is shown in Fig. II.

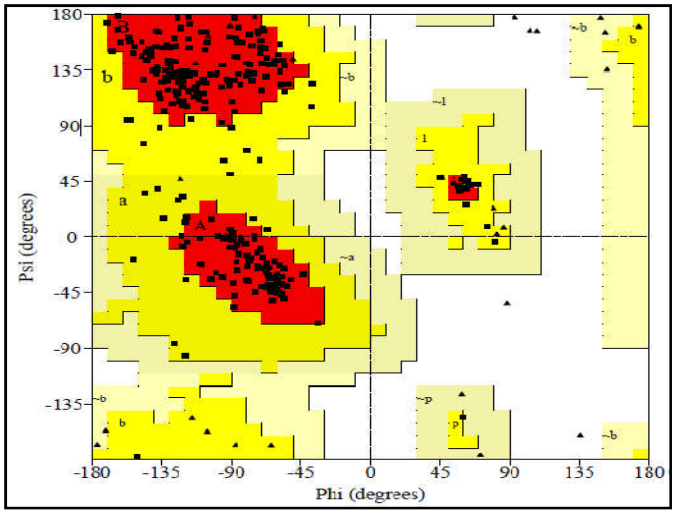


Fig. I. Ramchandran plot of Predicted 3D structure of PME

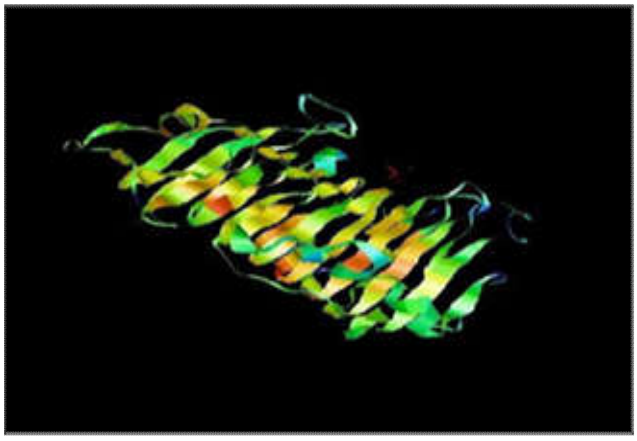


Fig. II. Ramchandran plot of Predicted 3D structure of PME

Table I: Residue no. & its % in different regions of Ramchandran plot of PME

Sr. no.	Regions	Residue no.	%
1.	most favoured regions [A,B,L]	236	86.1%
2.	additional allowed regions [a,b,l,p]	38	13.9%
3.	generously allowed regions [~a,~b,~l,~p]	0	0
4.	disallowed regions	0	0

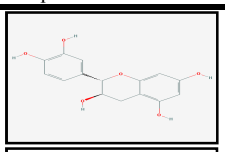
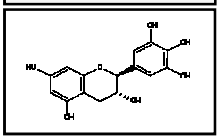
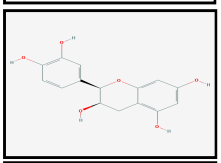
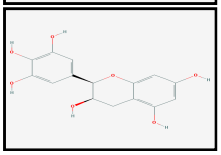
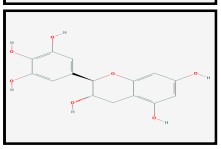
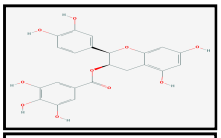
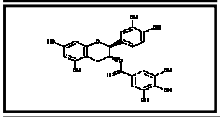
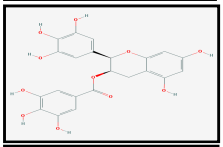
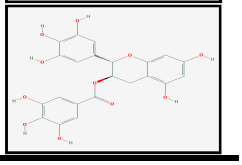
2. Active site prediction

Active site of PME was predicted by using CASTp server. 74 pockets were identified in PME molecule.

3. Designing ligand Library

Catechin compound was selected from PubChem as natural inhibitor for PME. The chemical compound was subjected to ZINC database to generate analogues for designing drug library. Total nine analogues were generated from ZINC database. The molecular descriptors were calculated and visualized by MarvinView package which shown in Table II.

Table II: Catechin analogues generated in ZINC database & their structures visualized in MarvinView package

S. No.	ZINC ID	Name of Compound	Structure of the compounds
1.	4214953	Catechin/ Cianidanol	
2.	3978494	2-(3,4-Dihydroxyphenyl)chroman-3,5,7-triol	
3.	14436215	Epicatechin	
4.	4214953	Epigallocatechin	
5.	3870337	(2S,3R)-2-(3,4,5-Trihydroxyphenyl)chroman-3,5,7-triol/ Gallocatechin	
6.	4534390	Epicatechin gallate	
7.	6040160	3- Galloylcatechin	
8.	3870412	Epigallocatechin gallate	
9.	3870413	Gallocatechin gallate	

4.Toxicity prediction of ligands

The ADME and Toxicity (Absorption, Distribution, Metabolism, and Excretion) properties of the chemical compounds were predicted using admetSAR server. The ADMET properties were Blood-Brain Barrier (BBB) penetration, HIA (Human Intestinal Absorption), and AMES toxicity were calculated. The predicted ADMET data were summarized in Table III.

Table III: Predicted ADMET properties of generated analogues

S.No.	Ligand Compounds	Blood-Brain-Barrier	Human intestinal absorption	P- glycoprotein inhibitor	CYP450 family	AMES toxicity	Carcinogenicity	Acute oral toxicity
1.	Catechin	BBB-	HIA-	Non-inhibitor	Non-inhibitor	Non- AMES toxic	Non- carcinogens	IV
2.	2-(3, 4 Dihydroxyphenol) chroman 3, 5, 7- triol	BBB+	HIA+	Non-inhibitor	Non-inhibitor	Non- AMES toxic	Non- carcinogen	II
3.	Epicatechin	BBB-	HIA+	Non- inhibitor	Non- inhibitor	Non- AMES toxic	Non- carcinogens	IV
4.	Epigallocatechin	BBB-	HIA+	Non-inhibitor	Non-inhibitor	Non- AMES toxic	Non- carcinogens	IV
5.	(2S,3R)-2-(3,4,5-Trihydroxyphenyl)chroman-3,5,7-trio	BBB-	HIA+	Non-inhibitor	Non-inhibitor	Non- AMES toxic	Non- carcinogens	IV
6.	Epicatechin gallate	BBB-	HIA+	Non-inhibitor	Non-inhibitor	Non- AMES toxic	Non- carcinogen	IV
7.	3- Galloylcatechin	BBB-	HIA+	Non-inhibitor	Non-inhibitor	Non- AMES toxic	Non- carcinogens	IV
8.	Epigallocatechin Gallate	BBB-	HIA+	Non-inhibitor	Non-inhibitor	Non- AMES toxic	Non- carcinogen	IV
9.	Gallocatechin Gallate	BBB-	HIA+	Non-inhibitor	Non-inhibitor	Non- AMES toxic	Non- carcinogen	IV

Table IV: Docking scores of each generated analogue with the PME

S.No.	Compounds	E –value
1.	Catechin	-233.20
2.	Dihydroxy phenyl chromne	-246.08
3.	Epicatechin	-233.20
4.	Epigallocatechin	-232.38
5.	(2S- 3R)- 2- (3, 4, 5- Trihydrxy phenyl chroman) 3, 5, 7- triol	-238.56
6.	Epicatechin gallate	-285.53
7.	3 galloylcatechin	-281.83
8.	Epigallocatechin gallate	-238.73
9.	Gallocatechin gallate	-238.73

5.Molecular Docking

Molecular docking studies are computational techniques for exploration of possible binding mode of a ligand (Catechin) to a given receptor (PME). Docking results of the catechin and its analogues using Hex molecular docking suite which reveals that interaction between the ligand and receptor. Eight analogues and catechin were docked with PME receptor and binding energy was calculated in Table IV. As per the table binding energy score of 3- Galloylcatechin (Zinc_6040160 (-281.83)) & Epicatechin gallate (Zinc_4534390 (-285.53)) is better as compared to other derivatives and Catechin molecule Zinc_4214953 was more compatible for the potential inhibitor with receptor than other ligand molecules. Further the docked complex between the Epictechin gallate (Zinc_3870412 (-285.53)) and PME molecule were visualized and shown in Figure 3. The below figure III reveals blocking of interacting residues Trp530, Arg503, Trp505, Asp413, Gln412, Asp465, Asp434 & Phe437 of PME with Epicatechin gallate (Zinc_4534390 (-285.53)).

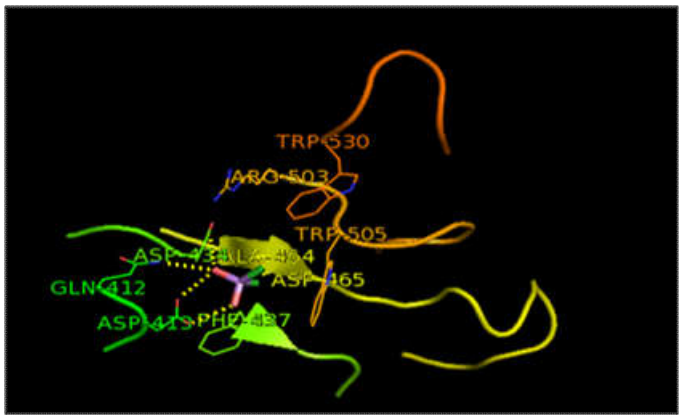


Fig. III: Docked complex between the Epictechin gallate (Zinc_3870412 (-285.53)) and PME molecule were visualized in Pymol

Conclusion

The tremendous growth of the weed plants & poisonous plants causes various problems to the habitat of the organisms present in that area hence their control is very essential hence we have aimed to identify the inhibitors of the enzymes playing importnat roles in their growth & developement. In the present in-silico study we have identified the novel inhibitor to the PME enzyme of the *A. thaliana* to stop the seed germination process by using the *in-silico* approaches. In the molecular docking step we have found that the two analogues of the catechin (natural inhibitor of the PME) Epicatechin gallate (Docking score= 285.53) & 3- Galloylcatechin (Docking score= 281.83) best than the other catechin analogues. The further in- vitro inhibtion study of the PME by these analogues on the other weeds & poisonous plants can provide the new alternatives to control their growth.

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