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Original Article

EXPLORING THE COMPETENCE OF PHYTOCHEMICAL COMPOUNDS TO COMBAT ANTHRACNOSE DISEASE OF CUCUMBER -AN *IN SILICO* APPROACH

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ABSTRACT

Objective: Anthracnose is one of the diseases of cucumber caused by the fungus *Colletotrichum lagenarium.* The enzymes of fungal melanin biosynthesis pathway act as a potential target for the synthetic fungicides. The present study aims to identify the rational phytochemical compound that exhibits inhibitory activity towards a key enzyme scytalone dehydratase (SCD) of melanin biosynthesis pathway.

Methods: Phytochemical compounds with antifungal activity were screened from the conventionally used medicinal plants and docked with the target enzyme SCD.

Results: The interaction amongst phytocompounds and the target SCD were evaluated. Five out of 20 compounds exhibited higher binding affinity compared to that of synthetic fungicide carpropamid.

Conclusion: The current study revealed that the compounds exhibiting significant interaction with SCD might act as an efficient fungicide for the control of anthracnose disease of cucumber.

Keywords: Anthracnose, *Colletotrichum lagenarium*, Melanin, Scytalone dehydratase, Phytochemical.

INTRODUCTION

Cucurbits are economically important plant crops that belong to the family Cucurbitaceae and this family consist of 118 genera and 825 species [1]. In India, a number of major and minor cucurbits are cultivated in the tropic and subtopic regions. Anthracnose is one of the destructive diseases seen in cucurbits family. Cucumber, watermelon and other types of melon are more prone to this disease [2]. The symptoms of this disease include dark-coloured spots or slightly sunken lesions on the leaves, stems and fruits of the plant [3].

Anthracnose disease is caused by the fungus *Colletotrichum lagenarium*. The fungal infection instigate with the conidial attachment followed by the formation of specialized structure called appressoria [4]. Appressorial cell wall contains melanin, which develops turgor pressure inside the appressoria and provides the mechanical strength to the fungus that invades into the host plant [5, 6]. The mutation study on melanin biosynthesis pathway of *Colletotrichum lagenarium* resulted in colourless appressoria that could not penetrate into the host plant [5]. In case of coffee berry disease caused by *Colletotrichum kahawae,* melanin biosynthesis inhibition resulted in reduction of turgor pressure inside the appresorium along with the fungal virulence [7].

Melanin biosynthesis takes place by means of DHN pathway in *Colletotrichum lagenarium*. Five molecules of malonyl CoA act as the precursor for the synthesis of melanin [8]. Melanin biosynthesis is a five step process where the initial step is the formation of 1,3,6,8 tetra hydroxy naphthalene from malonyl CoA this step involves the enzyme polyketide synthase. Later on, sequential reduction and dehydration results in the intermediates such as scytalone,1,3,8-trihydroxynaphthalene(1,3,8-THN), vermelone and1, 8 dihydroxy naphthalene (1,8 DNH). This on polymerization results in melanin formation. The fungal melanin biosynthesis pathway involves the enzymes such as tetrahydroxy naphthalene reductase, scytalone dehydratase and trihydroxy naphthalene reductase [9].

The fungal melanin biosynthesis pathway was found to be common in *Colletotrichum lagenarium* and *Pyricularia oryzae* [10]. The scytalone dehydratase (SCD) enzyme is one of the key enzyme which involves in the dehydration of scytalone to 1, 3, 8 trihydroxy naphthalene reductase. Cloning of *SCD1* gene of *Colletotrichum lagenarium* revealed that this particular enzyme was important for melanin biosynthesis pathway. The active site residues of SCD including Tyr-27, Asp-28, Tyr-47, His-82, Val-105, His-107, Ser-126, and Asn-129 were found to be conserved among the fungal species *Colletotrichum lagenarium* and *Pyricularia oryzae* [11]. Tricyclazole, pyroquilon and carpropamid are the fungicides used to control the disease caused by *Colletotrichum lagenarium* [12]. Synthetic fungicides play a major role in the fungal disease management. However it holds disadvantages such as development of resistance to phytopathogen, expensive and non- biodegradable [13]. Hence there is an urge for the development of potential eco-friendly fungicide which can manage the disease efficiently. The bioactive compounds belongs to conventionally used medicinal plants such as *Glycyrrhiza uralensis Fisch*, *Zingiber officinale Roscoe*, *Juniperuschinensis*, *Zanthoxylum piperitum*, *Toddalia asiatica*, *Cinnamomum zeylanicum*, *Paeonia moutan Sims*, *Azadirachta indica*, *Calotropis gigantean*, *Piper betle*, *Atalantia monophylla*, *Tinospora cordifolia* and *Eugenia aromatica* were reported for antifungal activity [13, 14, 15, 16]. The mode of action of numerous antifungal compounds remains unclear. In the present study we selected 20 phytochemical compounds with antifungal activity and the potential inhibitor for the target SCD of *Colletotrichum lagenarium* were identified by *in silico* molecular docking approach

MATERIALS AND METHODS

Target sequence retrieval

The enzyme scytalone dehydratase was found to play a key role in the melanin biosynthesis pathway of the fungus *Colletotrichum lagenarium* [11]. The sequence of SCD was retrieved from the Uniprot database (Accession no: Q00455) [17] and it has 188 aminoacid residues with the molecular weight of 21,687 Dalton. The three dimension structure of the enzyme scytalone dehydratase was not yet determined. Hence the structure was modelled for further interaction studies.

Template Selection

A sequence similarity search tool BLAST was used to select the template which has high similarity to the target protein [18].

BLAST search was performed against the protein structure database (PDB) with default parameters. Template was selected based on the identity, similarity, BITS score, query coverage and E value between target and template sequences.

Computational modelling and active site prediction

The three dimensional structure of SCD in *Colletotrichum lagenarium* was modelled by using Modeller 9.13 [19]. The structures of the target and template were superimposed and root mean square deviation (RMSD) value was generated using PyMol tool [20]. In order to obtain the stable structure and to minimize the steric clashes energy minimization was done for the modelled structure using SPDBV [21] and the modeled structure was also validated by SAVES server [22]. Ramachandran plot was analyzed to verify the amino acids present in the allowed and disallowed regions. The active site of the SCD was identified using Q-Site Finder. It predicts the active sites based on the interacting energy between the protein and a simple vander waals probe to locate energetically favourable binding sites [23].

Ligand generation and docking

The structures of phytochemical compounds from the traditionally used medicinal plants were retrieved from the PubChem database [24]. The small molecule structures were downloaded and used for docking studies. The molecular docking analysis was done using GLIDE (Grid Based Ligand Docking with Energetics) a ligand receptor docking suite from Schrodinger software. The glide docking involves a series of steps such as protein preparation, ligand preparation, grid generation and docking. The target structure was prepared by means of protein preparation wizard where the structure was refined using OPLS force field and the grid generation was carried out for the prepared protein by specifying the active site residues. In addition, the ligand structure was prepared by using LigPrep module and finally extra precision (XP) docking was performed.

RESULTS AND DISCUSSION

Homology Modelling and structure validation

The structure of the target protein SCD was generated by using the template scytalone dehydratase with the inhibitor cyanocinnoline (3STD) of *Magnoporthe grisea*. The chain A of the template protein showed 72% sequence similarity and the significant e-value (1e-91) with the target protein. The stereo chemical similarity between the target and template was obtained by calculating the RMSD. The calculated RMSD value was about 0.254A^{0}.

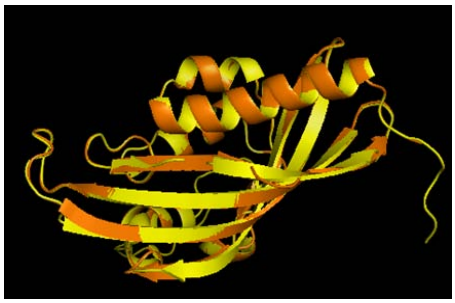


Fig.1: The superimposed structure of modelled protein scytalone dehydratase. The template (scytalone dehydratase with the inhibitor cyanocinnoline) and the target (scytalone dehydratase) were showed in yellow and orange colour, respectively.

The RMSD value suggested that the modelled structure was highly similar to the template protein. The superimposed structure of target and template was shown in Fig 1. Furthermore, the modelled structure was validated using SAVES server and the generated Ramachandran plot revealed that 92.9% of aminoacid residues were found in the allowed region and no residue was seen in disallowed region (Fig.2).

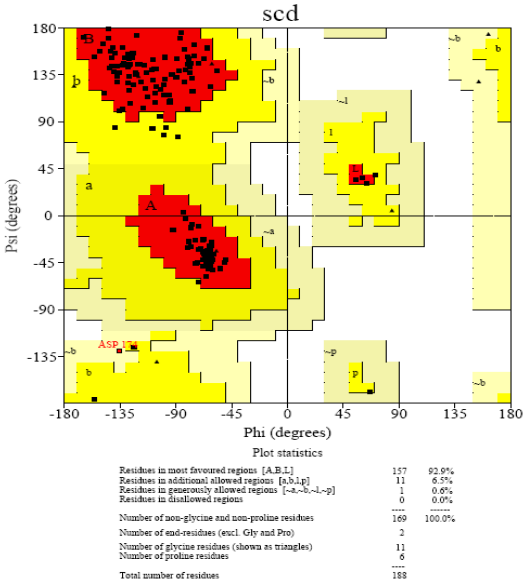


Fig. 2: The Ramachandran plot for the modeled protein scytalone dehydratase

Binding pocket analysis

The predicted active site residues are Tyr-27, Asp-28, Tyr-47, His-82, Val-105, His-107, Ser-126, and Asn-129. These residues found to relay with that of previous report deals with the residue conservation of SCD among *Magnoporthe grisea* and *Colletotrichum lagenarium* [11].

Screening of phytochemical compounds

Furthermore, the phytochemical compounds from the traditionally used medicinal plants with antifungal activity were selected based on the literature survey [13, 14, 15, 16] and its listed in Table1. The 3D structure of the ligands were retrieved from the PubChem database and subjected to docking studies.

Molecular Docking analysis

The screened phytochemical compounds were docked with the modelled target SCD of *Colletotrichum lagenarium*. Totally we found 12 out of 20 phytochemical compounds showed interactions with the active site residues of the targeted protein. The commercially used fungicide carpropamid was used as a reference compound and also docked with the enzyme SCD.

The phytochemical compounds such as vitrofolal F, atalaphylline, licochalcone A, buxifoliadine A, gingerol, sanshool, carvacrol, citronellol, eugenol, paeonol, calotropin and calactin found to interact with the active site residues of SCD. The glide score, number of H-bonds and the interacting residues of the phytochemical compounds along with the synthetic fungicide were shown in Table.2.

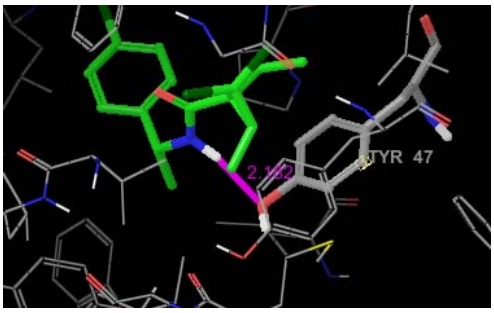


Fig. 3: Interaction of the synthetic fungicide carpropamid with the target SCD

The molecular interaction revealed that five phytochemical compounds have greater binding affinity with the target SCD than the synthetic fungicide. The interactions of those phytochemical compounds with the target SCD was given in Fig.4. Vitrofolal F from the plant *Vitex negundo* exhibit significant binding affinity with the glide score -11.11 Kcal/mol. Two interactions were observed with the active site residues PHE 50 and HIS 82. The interactions were shown in Fig.4 (a). The compounds atalaphylline, buxifoliadine A resulted in single interaction with the residue HIS82 and the glide score was -10.23 and -8.99 Kcal/mol respectively.

The interaction of these compounds with the residue HIS82 was shown in the Fig. 4(b), Fig. 4(d). These compounds were derived from the plant species *Atalantia monophylla.* The compound licochalcone A from the plant *Glycyrrhiza uralensis Fisch* resulted in the glide score of -9.54 Kcal/mol and single interaction was observed with ASN128 which was represented in Fig.4(c). Gingerol from the plant *Zingiber officinale* Roscoe holds the glide score -8.32 Kcal/mol and it interacted with two active site residues (TYR 27 and TYR 47) of the target SCD, interactions were shown in the Fig.4 (e).

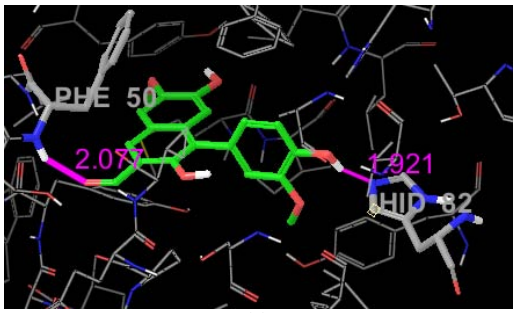
Table 1: List of antifungal phytochemical compounds

Compound	Plant Name	Plant part
Curcumin	*Curcuma longa*	Rhizome
Gingerol	*Zingiber officinale Roscoe*	Rhizome
Licochalcone A	*Glycyrrhiza uralensis Fisch.*	Root
Nootkatin	*Juniperuschinensis*	Fruit
Sanshool	*Zanthoxylum piperitum DC.*	Fruit
Eugenol	*Eugenia aromatic*	Flower bud
Cinnamic aldehyde	*Cinnamomum zeylanicum*	Bark
Paeonol	*Paeonia moutan Sims*	Bark
Salannin	*Azadirachta indica*	Seeds
Calactin	*Calotropis gigantea*	Leaf
Calotropin	*Calotropis gigantea*	Leaf
Carvacrol	*Piper betle*	Leaf
Camphene	*Vitex negundo*	Leaf
Vitrofolal F	*Vitex negundo*	Roots
Atalaphylline	*Atalantia monophylla*	Roots
Buxifoliadine A	*Atalantia monophylla*	Roots
Citrusinine I	*Atalantia monophylla*	Roots
Tembetarine	*Tinospora cordifolia*	Stem
Citronellol	*Toddalia asiatica*	Fruit
Nitidine	*Toddalia asiatica*	Roots

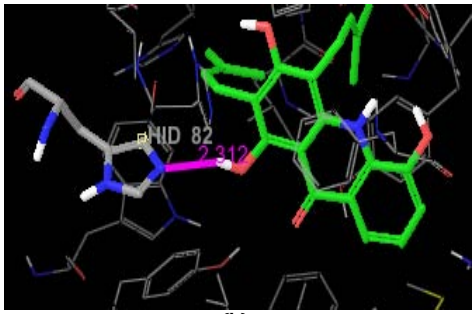
Table 2: Phytochemical compounds and the interacting residues of SCD

Bioactive Compound	Number of H-bonds	Interacting residue	Glide score Kcal/mol
Vitrofolal F	**2**	**PHE 50,HIS 82**	**-11.11**
Atalaphylline	**2**	**HIS82**	**-10.23**
Licochalcone A	**1**	**ASN128**	**-9.54**
Buxifoliadine A	**1**	**HIS 82**	**-8.99**
Gingerol	**2**	**TYR47,TYR27**	**-8.32**
Sanshool	1	TYR47	-7.51
Carvacrol	1	TYR47	-7.09
Citronellol	1	TYR27,47	-6.35
Eugenol	1	HIS82	-6.32
Paeonol	1	TYR47	-5.4
Calotropin	1	GLN108	-2.43
Calactin	1	GLN81	-1.48
Synthetic Fungicide			
Carpropamid	1	Tyr47	-8.71

The fungicide carpropamid exhibit single interaction with Tyr47 of SCD and the possessed glide score was -8.17 Kcal / mol. The interaction of the fungicide and the target was shown in Fig.3.



(a)



(b)

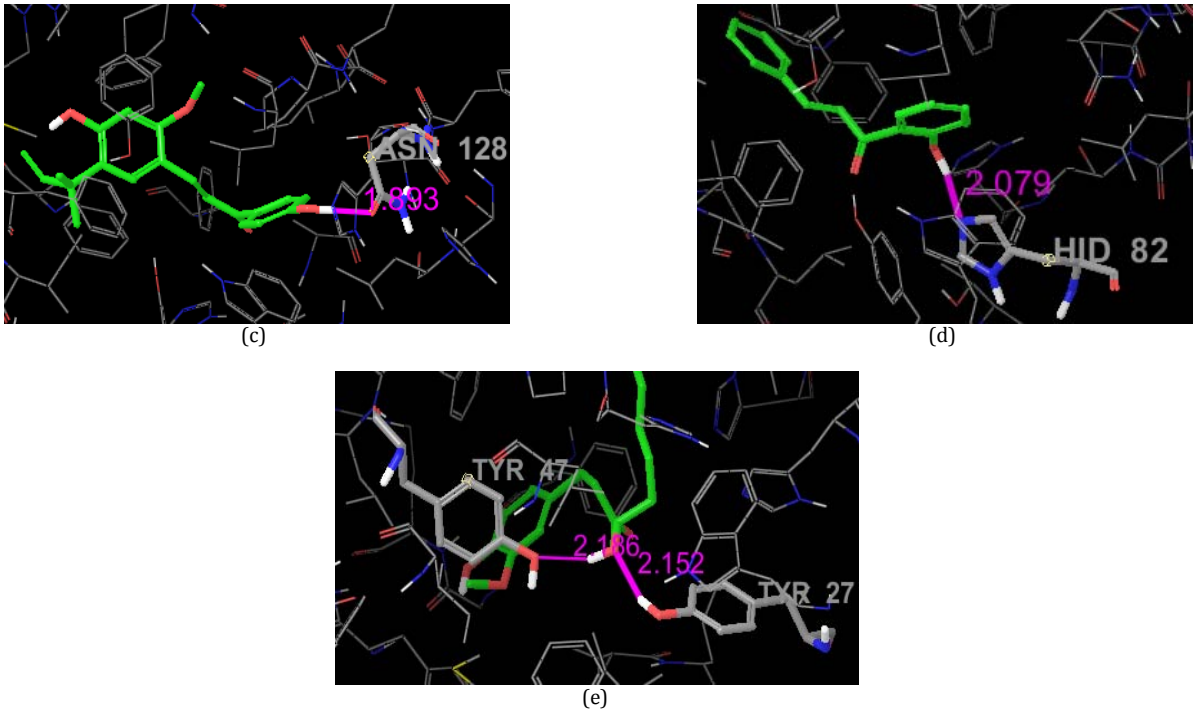


Fig. 4: Complex structure of SCD with phytochemical compounds a) vitrofolal F, b) atalaphylline c) licochalcone A d) buxifoliadine e) gingerol

This preliminary docking studies of 20 phytochemical compounds from conventionally used medicinal plants with the target SCD resulted in five best phytochemical compounds such as vitrofolal F, atalaphylline, licochalcone A, buxifoliadine A and gingerol from the medicinal plants *Vitex negundo, Atalantia monophylla, Glycyrrhiza uralensis Fisch* and *Zingiber officinale* Roscoe. These five compounds exhibited higher binding affinity with the active site residues of the target SCD compared to that of synthetic fungicide carpropamid.

CONCLUSION

There is an urge to find alternate for the synthetic fungicides because of the cost and side effects. The present comparative docking analysis study revealed the efficiency of certain phytochemical compounds that can be used to control anthracnose of cucumber. The structure of these phytochemical compounds would act as a prototype for the synthesis of efficient fungicides.

CONFLICT OF INTERESTS

Declared None

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