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Insilico analysis of cranberry proanthocyanidin catechin-(4alpha-2)-phloroglucinol as an inhibitor for modeled afimbrial adhesin virulence protein of uropathogenic *Escherichia coli*

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ABSTRACT

Fimbrial adhesion is a Virulence Determinant which is classified under Adhesins category of virulence factor of uropathogenic *Escherichia coli.* Afimbrial adhesin Protein with swissprot accession number P12730, of length 181 amino was selected for modeling using Bioinformatics tools. Modelled protein has been submitted to protein model database and has been assigned an accession number of PM0075877. Docking analysis of Catechin-(4alpha-2)-phloroglucinol from cranberry against modelled fimbrial adhesion was carried out using hex docking tool. Some of the commonly used antibiotics to treat urinary tract infections caused by Uropathogenic *E.coli* which includes Oflaxacin, sulfamethoxazole, Trimethoprim were subjected to docking analysis for comparative studies.

KEYWORDS

Uropathogenic *E-coli;*
Fimbrial adhesion;
Catechin;
Docking analysis.

INTRODUCTION

Urinary tract is the second most common site of bacterial infection in humans and thus represents a major source of human discomfort. It is now recognized that there are subsets of faecal *E.coli,* which can colonize periurethral area, enter urinary tract and cause symptomatic disease. These are currently defined as uropathogenic *E.coli*[1]. Uropathogenic *Escherichia coli* (UPEC) are the major causative agent of urinary tract infections (UTIs)[2]. UTIs cause significant medical expenditures reaching $1.6 billion each year in the United States[3]. Clinically, UTIs are considered acute, self-limiting infections despite the prevalence of recurrent symptoms two or more times within months of a primary infection[4]. In humans; the disease is associated to many virulence factors present in the uropathogenic *E.coli* (UPEC), including haemolysin, aerobactin, adhesins, serum resistance, cytotoxic necrotizing factor (CNF), etc.[5].

The specific attachment of bacteria to mucosal surfaces of animal tissues is gaining increasing attention, as it is considered to be a prerequisite to colonization of the host in the pathogenesis of bacterial infections[6]. The adhesion is generally mediated by fimbriae, which recognize cell surface carbohydrate structures on the epithelial cells[7]. Fimbriae are one of the primary mechanisms of virulence for E. coli.

The fimbriae produce 2 adhesins (mannose sensitive and mannose resistant) that attach to receptors on uroepithelial cells[8]. Their presence greatly enhances the bacteria's ability to attach to the host and cause disease[9]. Many studies have shown that bacterial adherence is an essential virulence factor in the patho-

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genesis of community acquired Urinary Tract Infections[10].

In the late 1970s it was recognized for the first time that *E.coli* strains causing urinary tract infections typically agglutinate human erythrocytes despite the presence of Mannose and this was mediated mainly by fimbriae[11]. SubseThere are many studies which state that cranberry compounds are effective against UTI's[13]. This effect is achieved by inhibiting the infecting bacteria, *Escherichia coli*, from adhering to uroepithelial cells[14]. Cranberries contain a unique polymeric compound known as proanthocyanidin[15]. Proanthocyanidin shows a very strong inhibitory activity against mannose-resistant adhesins produced by urinary isolates of *E.coli*[16].

The antiadhesive property of cranberries probably helps to prevent UTI in 2 ways:

1) It directly prevents *E.coli* from adhering to uroepithelial cells and
2) It selects for less adherent bacterial strains in the stool.

A recent study showed that regular consumption of cranberry juice was also effective in cases in patients with UTI caused by antibiotic-resistant bacteria[17]. Recently, proanthocyanidin extracts were isolated from cranberries by bioassay-directed fractionation and exhibited antiadherence activity against uropathogenic P-fimbriated
E.coli[18].

Cranberry proanthocyanidins have some unique features that distinguish them from other types of proanthocyanidins, and may ultimately be the reason for their in-vivo antiadhesion effects[19].

MATERIALS AND METHODS

Protein with Swiss-Prot primary accession number P12730, of length 181 amino acids was selected for modeling of Afimbrial adhesin AFA-I.

Modelling

Modelling of protein refers to constructing an atomic-resolution model of the "target" protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein ("template"). Modelling of target protein was carried out using Swiss-PdbViewer (or SPDBV).The template molecule with maximum percentage identity, having a PDB accession number 2JTYA, was selected for modelling of the target protein. The amino acid sequence of the target to be modelled and the template sequence is provided. The sequence alignment and template structure are then used to produce a structural model of the target.

Inhibitor molecule

Catechin-(4alpha-2)-phloroglucinola Proanthocyanidin from cranberry was selected as lead molecule for Insilco analysis of its inhibition activity against modelled fimbrial adhesion.

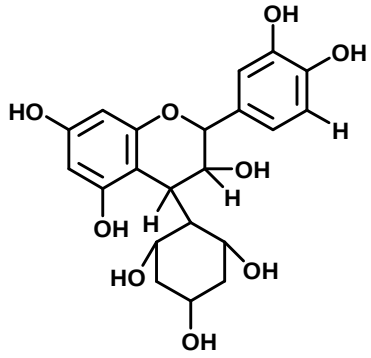

Figure 1 : Catechin-(4alpha-2)-phloroglucinol a Proanthocyanidin from cranberry

Lead validation

Validation assay for the lead molecule was carried out using OSIRIS Property Xplorer.

Docking studies

Docking analysis was carried out using Hex 5.1. Analysis of Catechin-(4alpha-2)-phloroglucinol a Proanthocyanidin from cranberry as an inhibitor against modelled fimbrial adhesion was carried out using Hex 5.1. Further Hex 5.1 was also used to visualize Interaction of Catechin-(4alpha-2)-phloroglucinol with modelled fimbrial adhesion protein. Some of the commonly used antibiotics to treat urinary tract infections caused by Uropathogenic *E.coli* which includes Oflaxacin, sulfamethoxazole, Trimethoprim were subjected to docking analysis for comparative studies using Hex 5.1.

RESULTS AND DISCUSSION

Modelled structure was obtained using SPDBV tool. This was used as the target protein for further analy-

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sis. Docking analysis of Catechin-(4alpha-2)-phloroglucinol a Proanthocyanidin from cranberry as an inhibitor against modeled fimbrial adhesion was carried out using Hex 5.1. Submitted modelled structure has been assigned an accession number PM0075877.This accession number can be used to retreive the submitted protein structure.

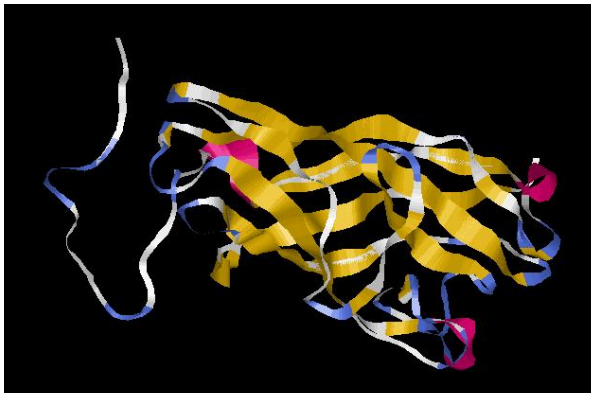


Figure 2 : Structure of modelled fimbrial adhesin protein.

Lead validation

Validation assay for the lead molecule was carried out using OSIRIS Property Xplorer. This involves validating the lead molecule based on their properties such as druglikeness, mutagenicity, toxicity, etc.

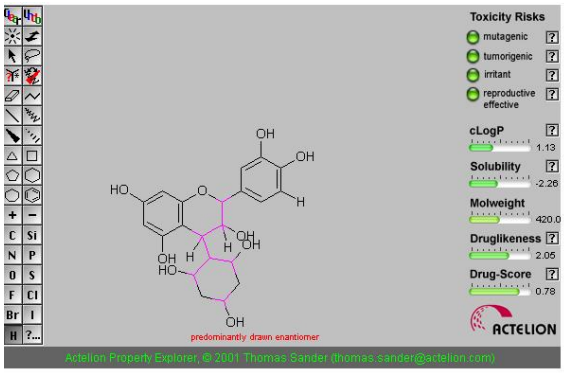


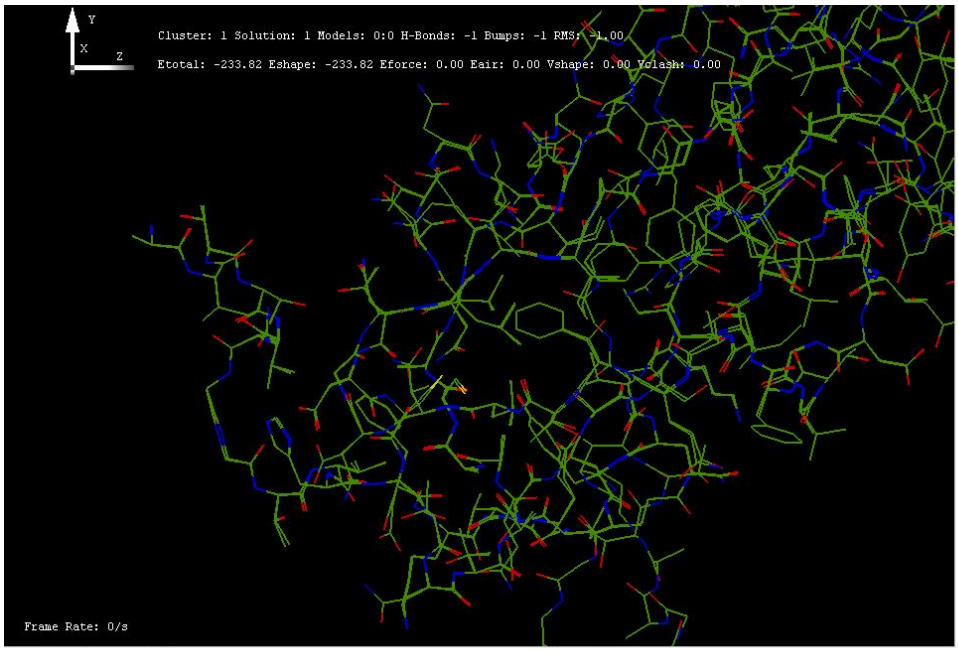
Figure 3: Result of validation assay.

It is observed that Catechin-(4alpha-2)-phloroglucinol clears mutagenecity, druglikeness, tumorogenic, irritant assays. Hence it can be considered as a potential inhibitor for further analysis.

Docking analysis

Docking analysis of Epicatechin-(4beta 2)-phloroglucinol a Proanthocyanidin from cranberry as an inhibitor against modelled fimbrial adhesion was carried out using Hex 5.1.

Interaction of Catechin-(4alpha-2)-phloroglucinol with the modelled target protein and its E Value is shown in Figure 4.



E Value: -233.82 kcal/mol

Figure 4 : Interaction of catechin-(4alpha-2)-phloroglucinol a proanthocyanidin from cranberry with modeled fimbrial adhesion virulence protein.

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Some of the most commonly used antibiotics such as Oflaxacin, Sulfamethoxazole and Trimethoprim, to treat Urinary Tract Infections were subjected to Docking Analysis to compare them with the lead molecule. The Binding Energy values obtained for the lead molecule and these antibiotics are shown in TABLE 1.

TABLE 1 : Comparison of E values between existing antibiotics to treat UTI and the lead molecule

Sl. No.	NAME OF THE COMPOUND	ΔG kcal/mol
	Lead Molecule	
1	Catechin(4-alpha-2)phloroglucinol	-233.82
	Commonly used Antibiotics	
1	Ofloxacin	-190.88
2	Trimethoprim	-183.28
3	Sulfamethoxazole	-170.00

CONCLUSION

Since its reported that Uropathogenic E Coli have developed antibiotic resistance(Gupta et al 2002), catechin Proanthocyanidin from cranberry with many evidences of its activity against virulence proteins of Uropathogenic *E.coli* has a very good prospective of being used as a medicine for Urinary tract infections caused by *Escherichia coli*. Comparative docking analysis of commonly used antibiotics used for treatment of urinary tract infections caused by Uropathogenic *E.coli* also suggest that catechin can be an alternative for the treatment.

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