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Computer aided Screening of Phytocompounds from *Trigonella foenum-graecum* (Fenugreek) Targeting Mutual Sub-cellular Proteins of *Bacillus subtilis* and *Escherichia coli*

Research Article

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Abstract

Objective: The prediction of bacterial sub-cellular proteins can be considered aid to biotechnological research. Bacterial proteins play a major role in cell division as well as in virulence. Here, we attempt to identify the potential phytocompounds *Trigonella foenum-graecum* from that can spot those bacterial sub-cellular proteins. **Methodology:** In-silico screening for phytoligands from Fenugreek with subjected to molecular docking simulations for better perception of correlation between phytocompounds and the target receptors. **Results:** Quercetin, Smilagenin, Diosgenin & Naringenin from Fenugreek possess immense binding affinity against subcellular bacterial proteins.

Conclusion: Based on all outcomes, we assertion that 4 phytoligands (Quercetin, Smilagenin, Diosgenin & Naringenin) could serve as an effective anti-bacterial compounds to cure bacterial infections.

Keywords: Phytocompounds, Sub-cellular proteins, Molecular docking, Fenugreek.

Introduction

Trigonella foenum-graecum (Fenugreek) is an annual plant native to the family of *Leguminosae*. Fenugreek has a momentous effect on filter the blood and help to detox the body. Many bacterial strains like *Escherichia coli, Pseudomonas aeruginosa, and Streptococcus pyogenes* gained attention for their capability to cause infection in lungs [1], Pharyngitis [2], and urinary tract infection [3]. In case of bacterial infection treatment, antibiotics play a major role to mitigate the infections. Antibiotics like sulphonamides, tetracycline, fluoroquinolones, Methicillin, and Penicillin have been utilised to stop the microbial cell wall formation [4]. Antibiotic resistance like Methicillin was first used in the 1963 employing *Methicillin Resistant Staphylococcus aureus* (MRSA).

Moreover, some studies clearly shows on antimicrobial resistance that the bacterial strains can also gain resistance in contrast to antimicrobial peptides [5,6]. The leaf extracts of ***Trigonella foenum-graecum*** **(**Fenugreek) is well known for its various type of pharmacological activities like anticancerous, antimicrobial, antifungal and antioxidant activities. The main group of compounds in Fenugreek is Flavonoids and saponin. This includes: Vitexin-7-O-glucoside, Isovitexin, Diosgenin, Luteolin, Naringenin, Sarsasapogenin, Isoorientin, Neotigogenin, Tricin, Orientin, Gitogenin, Quercetin, Smilagenin, Tigogenin, Vicenin-2, Vitexin, and Yamogenin [7]. Based on the chemical structure and ADME properties, mainly 9 phytocompounds have been isolated for study. From

the listed phytocompounds, 9 were selected (Diosgenin, Naringenin, Orientin, Quercetin, Smilagenin, Tigogenin, Vicenin-2, Vitexin, and Yamogenin). The given phytoligands were predicted to interact with 5 different proteins present in E.coli and B. subtilis. In this study, comparative investigation of selected phytocompounds from the plant ***Trigonella foenum-graecum***(Fenugreek) with the known bacterial protein receptors was carried out employing molecular docking studies which analysed the novelty of phytoligands.

Methodology

Retrieval of target proteins

Homology model structures of biological membrane proteins of strains *Bacillus subtilis* and *Escherichia coli* were fetched from PDB (Protein Data Bank) [8]. Target proteins assign the bond order, missing bonds & charges to the respective structures in case of preparation process and make them for molecular docking simulation.

Preparation of ligands

The 3-D structure of all phytocompounds of Fenugreek was fetched from PubChem database [9] for docking studies. During the preparation process, bond orders, hybridization, hydrogen explicit& energy minimized structures can be obtained. Based on the homology structural similarity, 9 phytocompounds of Fenugreek were targeted as ligands and subjected to ADMET analysis [10].

Pharmacokinetics of the ligand-protein interaction

According to pharmacokinetics, binding energy and correlation with receptors were investigated on the behalf of internal ES or Internal Electrostatic Interaction and aromatic bond interaction. Complex structure of the ligand-receptor interaction refers to hydrogen bond, hydrophilic and hydrophobic interaction, bond donors & receptors expose the pharmacokinetic features.

Excerpt of subcellular proteins

In this study, the antibiotic resistance of bacteria can be targeting its cellular proteins or cytoskeleton receptors like MreB and MreC. MreB is a bacterial sub-cellular protein that has been observed during actin homology [11]. MreB is a single cytoskeleton protein which is presents in *Escherichia coli* and they initiate the formation of helical structures which are responsible for rod shape of *Escherichia coli* [12]. MreB takes part in the formation of spatial organization of Penicillin binding proteins or receptor [13] which is based on peptidoglycan synthesis (Table 1-3).

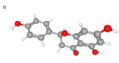
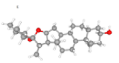
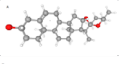
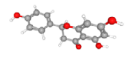
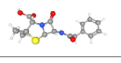
Table 1

Protein	Bacterial strains	References
ParM	*Escherichia coli and Bacillus subtilis*	[14]
FtsZ	*Escherichia coli and Bacillus subtilis*	[15]
ZapD	*Escherichia coli and Bacillus subtilis*	[16]
MreB	*Escherichia coli and Bacillus subtilis*	[17]
MreC	*Escherichia coli and Bacillus subtilis*	[18]

Table 2: Phytoligands with good ADME and Drug-likeness range.

Sr.no	Ligands	Molecular weight	LogP	Heavy atom count	Drug-likeness score
1	Diosgenin	414.6 g/mol	5.03	30	0.9
2	Naringenin	272.25 g/mol	1.84	20	0.64
3	Orientin	448.4 g/mol	-0.47	32	0.17
4	Quercetin	302.23 g/mol	1.23	22	0.2
5	Smilagenin	416.6 g/mol	5.24	30	0.89
6	Tigogenin	416.6 g/mol	5.24	30	0.76
7	Vicenin-2	594.5 g/mol	-1.71	42	0.11
8	Vitexin	432.4 g/mol	0.02	31	0.51
9	Yamogenin	414.6 g/mol	5.01	30	0.03

Table 3: Autodock scores (kcal/mol) for phytoligands with *Escherichia coli&Bacillus subtilis* subcellular protein targets.

Compound	3-D Chemical structure	Chemical formula	Bacterial protein receptor	Binding energy (kcal/mol)
Quercetin		$C_{15}H_{10}O_7$	MreB, MreC, ParM, ZapD, FtsZ	-89.4
Smilagenin		$C_{27}H_{44}O_3$	MreB, MreC, ParM, ZapD, FtsZ	-94.52
Diosgenin		$C_{27}H_{42}O_3$	MreB, MreC, ParM, ZapD, FtsZ	-103.4
Naringenin		$C_{27}H_{32}O_{14}$	MreB, MreC, ParM, ZapD, FtsZ	-111.5
Penicillin		$C_{16}H_{18}N_2O_4S$	MreB, MreC, ParM, ZapD, FtsZ	-112.3

MreC is another cytoskeletal sub-cellular protein which are also responsible for shape of a bacterial cells. Absence of MreC protein leads to the growth and morphological problems [19]. MreC is more complex than MreC. MreC also Controls the cell viability & even creates the membrane bound complex [20].

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ParM is a prokaryotic actin homologue and helps in the R1 plasmid to convey opposite ends of the cell before cytokinesis [21,22]. ZapD protein takes part in cell division in *E.coli* [20] and also stabilizes FtsZ assembly for formation of Z-ring [23]. ZapD protein is a small sub-cellular protein which attaches to the FtsZ filaments [24]. FtsZ is also prokaryotic homologue protein to tubulin protein [25]. ZapD is ATPase while FtsZ is a GTPase which is responsible for cell division in *B.subtilis* [26]. Moreover, many indistinguishable proteins like SepE, SepF, and Alp7A are cytoskeletal proteins present in bacteria like *Escherichia coli* and *Bacillus subtilis* [27].

Molecular docking simulation

The molecular docking was performed to analyse the inhibitory activity of selected phytocompounds against respective receptor proteins. The docking process was employing with Autodock [28] and PyRx [29] software. The parameters were all set to molecular surface structure with extended Vander wall forces. In case of grid resolution, 30 A was kept for grid generation and cavity assumed using Autodock software. All the selected phytocompounds or ligands were docked against the respective bacterial receptor proteins and the exact generated structure were fetched based on binding energy or affinity. The synergy between the receptor protein and phytoligands depends on the number of hydrogen bonds and binding energy.

All the potential phytoligands were screened for identification using virtual screening tool (PyRx). These types of filters eliminate the compounds with poor drug likeness.

Result and Discussion

Ligands

The 3-D structures of all selected phytocompounds or ligands were retrieved from PubChem databases. All these structures were subjected to molecular docking simulation. The selected phytoligands were fetched in the SDF format followed by the conversion in the PDB format using PyMol software [30]. Moreover, all the shape complementary process was employed with accumulating RMSD 4.0 for molecular docking calculations.

Molecular Docking simulation

The molecular docking was performed to understand the inhibitory activity of selected phytoligands in contrast to bacterial proteins. The docking starts with the double

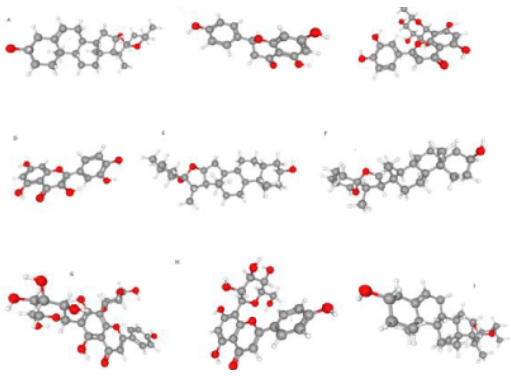

Figure 1: Lowest energy docked of protein receptor MreB with Quercetin and Smilagenin.

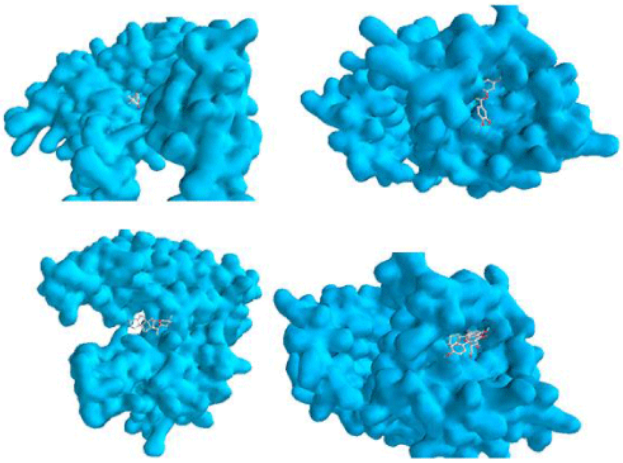

Figure 2: Lowest energy docked of protein receptor of MreC with Diosgenin and Nirangenin.

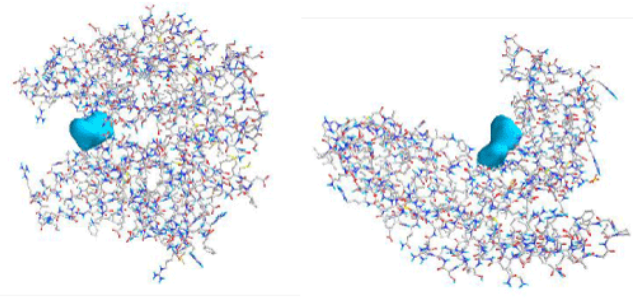

Figure 3: Lowest energy docked of protein receptor FtsZ with Quercetin & Diosgenin.

colored molecular surface according to their specific property to the bacterial proteins. And all the parameters

were set to the molecular surface with grid resolution 30A for grid generation using prediction algorithm [31]. The result of the docking mainly depends on the number of hydrogen bonds and binding energy (Figure 1-3).

The selected 9 phytocompounds from Fenugreek have been docked in contrast to bacterial receptor proteins and ligand contains around 4 flavonoids, 5 Glycosides, 6 lignin, and 9 long chain compounds. All selected phytocompounds were analysed to check the potential of compounds that has a capability to refer as a drug compound. All the collected phytoligands were subjected to ADME analysis using SWISSAdme online server, which assumes or predicts the ADME property of the phytoligands depends on their functional group, molecular structure, and properties like hydrogen bond donors, hydrogen bond acceptors and molecular weight, Polar Solvent accessibility. The compounds which found to be violate the ADME properties eliminated from them. In this study, only 9 phytocompounds were selected for further tests and risk assessment.

The compound toxicity test was based on the Lipinski's filter parameters. All the phytocompounds were non-carcinogenic in nature. So, all the selected phytocompounds were docked on the contrary to receptor proteins and best generated poses were collected based on the Autodock scores. Autodock and PyRx score are scoring function used to expose the binding affinity of docked poses. The correlation between the phytoligands and the bacterial proteins depends on the maximum hydrogen bonds and binding affinity or energy. In first docking mechanism, the phytocompounds Diosgenin and Naringenin were docked against *E.coli and B. subtilis* subcellular proteins and their docking score were compared to the co-crystal antibiotics. The already available antibiotic drug (Penicillin) was used as controls in the study. Both the phytocompounds and control drug were docked against subcellular bacterial proteins and phytocompounds with same binding affinity than the control drug were targeted.

The control drug, Penicillin exposes higher binding energy in contrast to protein FtsZ with Autodock and PyRx of -101.18 kcal/mol followed by Alp7A (-112.3 kcal/mol), and MreB (-109.42 kcal/mol). MreB expose higher binding affinity with phytoligands Quercetin and Smilagenin with docking score (-121.87 kcal/mol)& (-99.45 kcal/mol).

Fenugreek is known as 'Methi' throughout the India. Such type of plants has been observed to possess many medicinal properties like anti-oxidant, anti-fungal as well as cytotoxic activity. Fenugreek also possesses antimicrobial activity in contrast to *Vibrio cholera, klebsiella aerogenes, Escherichia coli, Proteus vulgaris, and Pseudomonas pyocyanea*. In this study, phytocompounds were docked against *E.coli & B.subtilis* bacterial subcellular proteins.

Conclusion

In silico analysis of Fenugreek phytocompounds was performed to determine the potential phytoligands with drug likeness. In this study, we observed that the phytocompounds Diosgenin, Quercetin and Smilagenin from Fenugreek possess great binding affinity on the contrary to the bacterial subcellular proteins with respective amino acid residues present in the active site of bacterial cell division & shape which classifying proteins like FtsZ, MreB, MreC, and Alp7A have exact docking scores. We believe that this work will help to find out the anti-bacterial analysis of phytocompounds from Fenugreek.

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