INNOVARE JOURNAL OF MEDICAL SCIENCES

Vol 10, Issue 4, 2022

ISSN - 2321-4406

Research Article

IN SILICO ANALYSIS OF SEAWEED PHYTOCHEMICAL FLAVONOID FOR THE INHIBITION OF STAPHYLOCOCCUS ENTEROTOXIN A

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Received: 01 May 2022, Revised and Accepted: 15 June 2022

ABSTRACT

Objective: The study is focused on evaluating the efficacy of seaweed flavonoids against staphylococcus enterotoxin A.

Methods: The drug-likeness and the pharmacological properties of the chosen ligand were evaluated using SWISS ADME. AutoDockTools-1.5.6 was used to carry out the docking analysis and determine binding parameters with molecular modeling. Docking visualization was performed using discovery studio.

Results: The bioactive flavones demonstrated high molecular interactions with staphylococcus enterotoxin A which assisted in its inactivation.

Conclusion: The study revealed that the phytocompounds chrysin and luteolin can be brought into play as a drug against staphylococcus enterotoxin.

Keywords: *Staphylococcus* enterotoxin A, Superantigen, *S. aureus,* Flavonoids, Molecular docking, Lipinski rule, Ramachandran plot.

 DOI: http://dx.doi.org/10.22159/ijms.2022v10i4.45116. Journal homepage: https://innovareacademics.in/journals/index.php/ijms

INTRODUCTION

The pharma industry has recently faced hurdles in synthesizing medications by addressing patterns of resistance identified in opportunistic microorganisms. Secondary metabolites of plants have grown in importance as medicinal agents over time. *Staphylococcus aureus* is a Gram-positive and cocci-shaped bacteria that clusters together [1,2]. *S. aureus* is known to trigger a variety of ailments including invasive infections and toxin-mediated illnesses. About 20% of persons are chronic carriers, 60% are intermittent carriers, and 20% are just occasionally carriers of *S. aureus*. *S. aureus* is commonly found in foods and is one of the most common causes of foodborne bacterial poisoning around the globe [3]. The high incidence of staphylococcal food poisoning is caused by inadequate pasteurization or decontamination of contaminated product sources. Poultry meat can be tainted during slaughtering or food preparation. Animal-borne strains can be spread to people by direct contact with animals or through the consumption of contaminated food [4,5].

The major sources of *S. aureus* in hospitals are septic lesions and carriage sites of patients and personnel. Carriage often precedes infection. The principal mode of transmission is through transiently contaminated hands of hospital personnel. Airborne transmission seems important in the acquisition of nasal carriage [6,7]. *Staphylococcus* enterotoxins are protein-based exotoxins. They are members of a family of more than 20 different staphylococcal and streptococcal exotoxins that are functionally related and share sequence homology. These toxins are produced by *S. aureus*. Staphylococcal enterotoxin molecules are relatively small and single-chain molecules with molecular masses of approximately 24-30kDa [5]. They share four major properties, that is, Superantigenicity, structural similarity, resistance to heat and proteolytic enzymes, and emetic properties [6]. Out of the 24 serologically distinct SEs, SEA is the most common serotype found in *S. aureus.* SEA and SEB are regarded as superantigens because of their ability to bind to class II MHC molecules on antigen-presenting cells and stimulate large populations of T cells that share variable regions on the β chain of the T cell receptor [2,8].

According to studies (−)-epigallocatechin gallate (EGCG), a phytochemical under phenolic acid could strongly bind to SEA which causes inhibition of the toxin [8]. The inhibition of this staphylococcus enterotoxin A (SEA) by the seaweed phytochemical flavones under the class of flavonoids would be the prime focus of this paper [9,10]. Flavones are a class of flavonoids based on the backbone of 2-phenyl chromen-4-one. The common phytochemicals in this class are apigenin, chrysin, and luteolin. Some of the seaweeds which contain flavonoids are *Corallina*, *Sargassum*, *Acanthophora,* and Jania [11,12]. According to studies, this class of flavonoids has efficient antibacterial properties against the *S. aureus.*

METHODS

Protein retrieval

The protein receptor FASTA sequence was procured from RCSB PDB and it was used to obtain the structure from the SWISS-MODEL template library [11]. The chosen receptor, that is, 1esf, from the SWISS-MODEL, was subjected to energy minimization using SwissPDBViewer (SPDBV) (Fig. 1)

Ligand preparation

The structure data file (SDF) of the chosen ligands apigenin, chrysin, and luteolin were obtained from PubChem (Fig. 2) [13-17] The conversion of SDF to PDB was done using PyMol [13]. Ligand cleaning is achieved using the Marvin Sketch [14].

Analysis of drug likeness of the ligand

SWISS ADME is considered to be a conventional drug discovery tool. All the properties of the chosen ligands were investigated using this tool. It includes Lipinski filter analysis, pharmacokinetic behavior, that is, blood-brain barrier, GI (Gastrointestinal) absorption, and the brain or intestinal estimated permeation method (BOILED-EGG) proposed [18,19].

Docking evaluation

Molecular docking of the ligands apigenin, chrysin, and luteolin with the 1esf receptor protein was achieved using Pyrx and AutoDockTools-1.5.6. The process of docking allows us to interpret the active binding site of each specific ligand along with its orientation. The entire receptor is

placed within the grid box to produce a blind docking with each ligand. The result generates nine outcomes consisting of binding affinity and RMSD values [18].

RESULT AND DISCUSSION

Ramachandran plot analysis

The Ramachandran plot shows the statistical distribution of the combinations of the backbone dihedral angles ϕ and ψ. ϕ values are plotted on the X-axis and the ψ values are presented on the Y-axis. Ramachandran's plot explains what kind of phi and psi angles are preferred in a particular secondary structure of the protein.The Ramachandran plot result exhibits that the majority of the amino acids in protein staphylococcus enterotoxin A are concentrated on the top left; this explains that they form beta sheets (Antiparallel) and the amino acids at the bottom depict the formation of the right-handed alpha-helical secondary structure. The most of the amino acids present in the two quadrants are considered highly preferred regions with a percentage of 91.5 (Fig. 3). Hence, this protein is acceptable.

Lipinski filter analysis

The drug-likeness was analyzed using a Lipinski filter (Table 1). It presents the pharmacokinetic properties, molecular mass, hydrogen bond donor, and bond acceptor of the selected ligand [19].

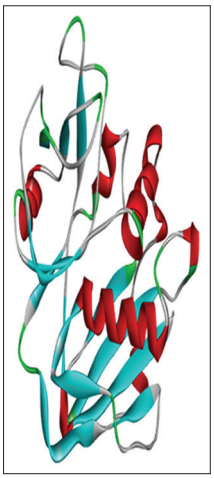

Fig. 1: 3-D structure of the protein

Criteria

Molecular weight in the range 250–300, H-bond donors ≤ 4, and H-bond acceptors ≤ 6. The selected ligands exhibit an acceptable range for human use. It also presents potential drug-like properties (Table 2).

Brain or intestinal estimated permeation method (BOILED-EGG)

The drug candidates are evaluated using the brain Or intestinal estimated permeation method (BOILED-EGG) [20]. It presents an accurate model of the selected ligand by computing its lipophilicity and polarity. The pharmacokinetics of the ligands specify brain and gastrointestinal (GI) permeation efficiency. High GI permeability and blood-brain barrier properties have been recorded in chrysin. Luteolin and apigenin unveil high GI permeability along with efficient solubility.

Docking results

PyRx

The outcomes generated through PyRx consist of nine values for each ligand. Chrysin (Figs. 4 and 5) and luteolin (Figs. 6 and 7) presented the least binding affinity value followed by apigenin. The binding affinity of chrysin and luteolin unfolds to be −8.4 followed by apigenin with the value of −8.2. These results were obtained by blind docking. The binding affinity results have helped to predict the possible active site for a ligand to bind to the protein to achieve inhibition.

Autodock analysis

Results of docking were interpreted using AutoDockTools-1.5.6 [18,21]. The RMSD table includes 10 outcome values for each specific ligand. AutoDock assists in analyzing the interactions of ligand molecules at the specified target site of the protein. The docking procedure was displayed for a rigid macromolecule and here the docking is faster than that of a flexible macromolecule. From the results of AutoDock, the binding sites in the protein for ligand interaction can be identified. Luteolin (Fig. 8) presents the least value of binding affinity (i.e., −7.08) followed by apigenin (i.e., −7.05) and chrysin (Fig. 9) (i.e., −6.44). High binding efficiency is displayed by the phytochemical luteolin (Table 3).

CONCLUSION

Based on *in silico* studies, the flavonoids chrysin and luteolin have presented favorable outcomes. Through SWISS ADME analysis, it is observed that chrysin shows high GI absorption along with BBB permeability and has moderate solubility whereas luteolin exhibits

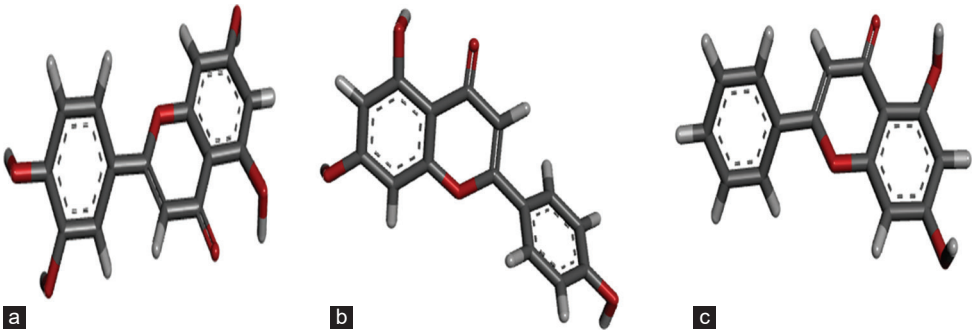

Fig. 2: Structure of ligands. (a) Luteolin, (b) Chrysin, (c) Apigenin

Table 1: Lipinski filter analysis

Phytochemicals	Molecular weight (g/mol)	Lipophilicity	Hydrogen atom donors	Hydrogen atom acceptors	Molecular Refractivity	Violoations
Apigenin	270.24	0.54	3	5	73.99	No violations, Obeys Lipinski
Chrysin	254.24	1.08	2	4	71.97	No violations, Obeys Lipinski
Luteolin	286.24	-0.03	4	6	76.01	No violations, Obeys Lipinski

Table 2: ADME analysis

Ligand	BBB permeability	GI absorption	Permeability glycoprotein substrate	Log S (Scale insoluble <-10<poorly<-6<Moderately <-4<Soluble<-2<very<0 < Highly)
Chrysin	Yes	High	No	−4.98
Luteolin	No	High	No	−3.82
Apigenin	No	High	No	−4.40

BBB: Blood brain barrier; GI: Gastrointestinal

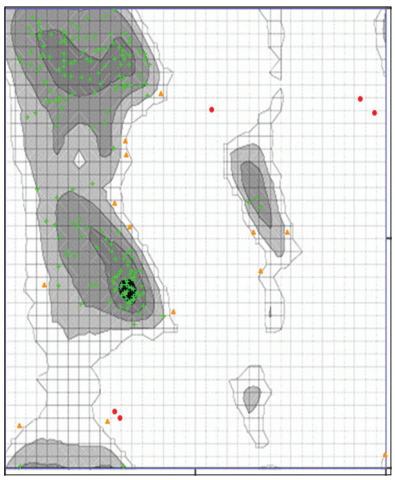

Fig. 3: Ramachandran Plot

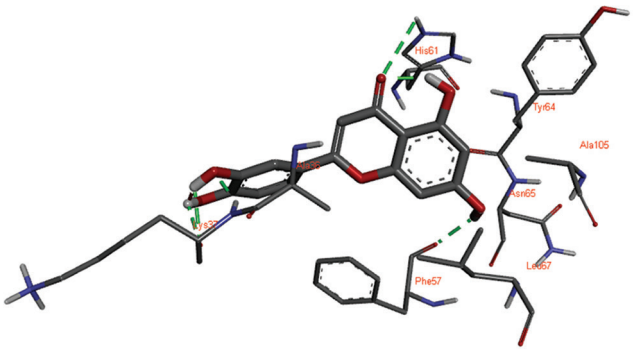

Fig. 4: 2-D chrysin protein interaction

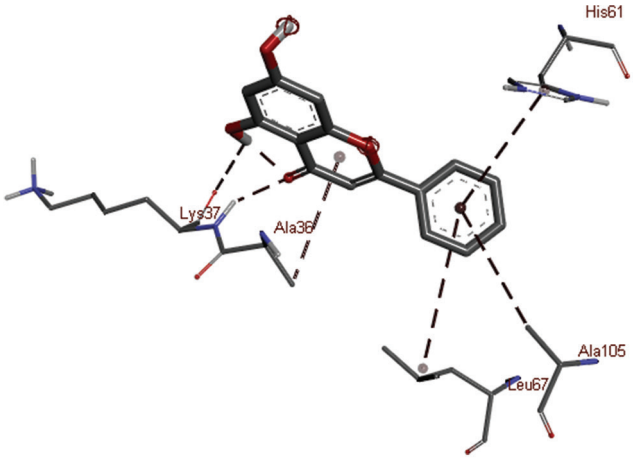

Fig. 5: Chrysin-protein interaction

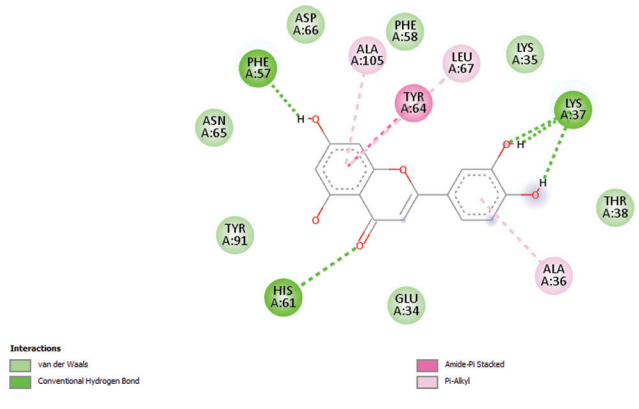

Fig. 6: 2-D structure of Luteolin protein interaction

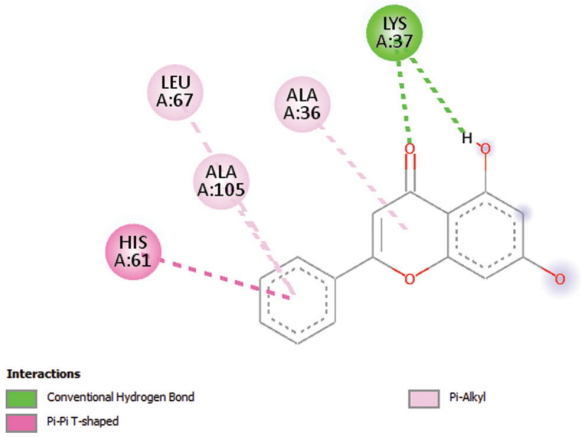

Fig. 7: Interaction of luteolin and protein

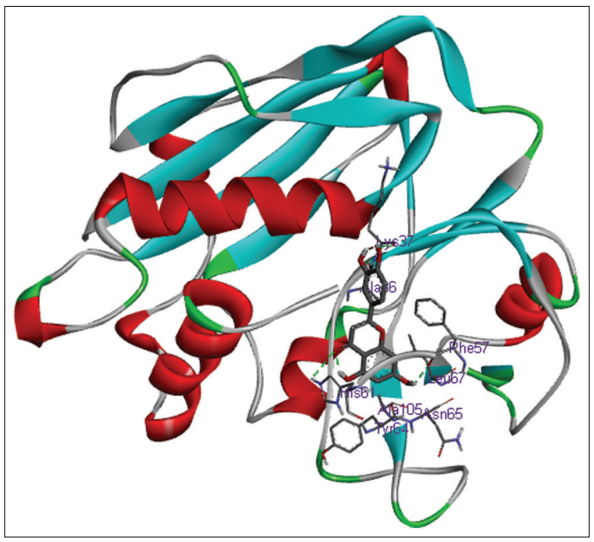

Fig. 8: 3-D view of Luteolin and protein interaction

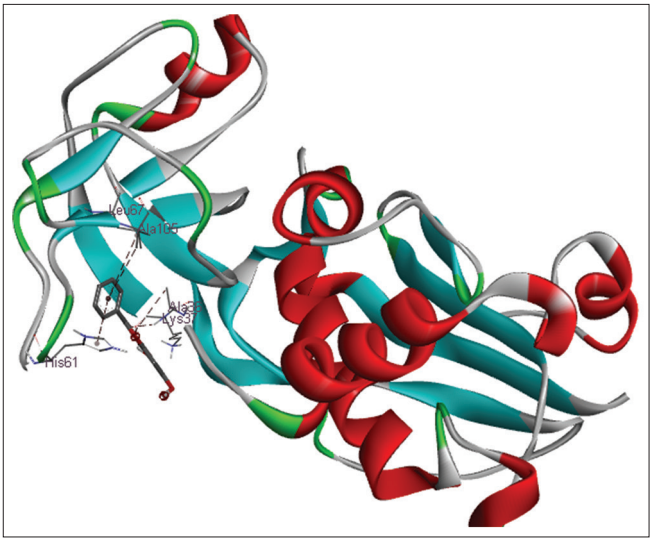

Fig. 9: 3-D chrysin protein interaction

Table 3: Results of docking

Ligand	Binding energy	Cluster RMSD	Reference RMSD	Grep pattern
Luteolin	−7.08	0.00	106.76	Ranking
Apigenin	−7.05	0.00	113.17	Ranking
Chrysin	−6.44	0.00	109.45	Ranking

high GI absorption and has solubility up to par. Docking analysis shows high-efficiency binding of the ligand (luteolin) to the receptor (Staphylococcus Enterotoxin A). Chrysin displayed a lower efficiency binding as compared to luteolin. Taking all these factors, it can be concluded that both chrysin and luteolin can be brought into play as a drug against staphylococcus enterotoxin.

ACKNOWLEDGMENT

We hereby acknowledge the sincere support and help extended by Research Team, BioNome Pvt. Ltd India, in carrying out the project work.

AUTHORS CONTRIBUTIONS

All the authors have equally contributed to the project.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

1. Kluytmans JA, Van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505-20.
2. Pinchuk IV, Beswick EJ, Reyes VE. Staphylococcal enterotoxins. Toxins (Basel) 2010;2:2177-97.
3. Hu DL, Nakane A. Mechanisms of staphylococcal enterotoxin-induced emesis. Eur J Pharmacol 2014;722:95-107.
4. Ortega E, Abriouel H, Lucas R, Gálvez A. Multiple roles of *Staphylococcus aureus* enterotoxins: Pathogenicity, superantigenic activity, and correlation to antibiotic resistance. Toxins (Basel) 2010; 2:2117-31.
5. Johnson HM, Russell JK, Pontzer CH. Staphylococcal enterotoxin microbial superantigens. FASEB J 1991;5:2706-12.
6. Bhunia AK. *Staphylococcus aureus*. In: Foodborne Microbial Pathogens: Mechanisms and Pathogenesis. New York, USA: Springer; 2018. p. 181-92.
7. Shimamura Y, Utsumi M, Hirai C, Nakano S, Ito S, Tsuji A, *et al.* Binding of catechins to staphylococcal enterotoxin A. Molecules 2018;23:1125.
8. Benedik E, Skrt M, Podlipnik Č, Ulrih NP. Binding of flavonoids to staphylococcal enterotoxin B. Food Chem Toxicol 2014;74:1-8.
9. Solberg CO. Spread of *Staphylococcus aureus* in hospitals: Causes and prevention. Scand J Infect Dis 2000;32:587-95.
10. Bortolaia V, Espinosa-Gongora C, Guardabassi L. Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. Clin Microbiol Infect 2016;22:130-40.
11. Schad EM, Zaitseva I, Zaitsev VN, Dohlsten M, Kalland T, Schlievert PM, *et al.* Crystal structure of the superantigen staphylococcal enterotoxin Type A. EMBO J 1995;14:3292-301.
12. Shelar PS, Reddy VK, Shelar GS, Kavitha M, Kumar GP, Reddy GV. Medicinal value of seaweeds and its applications-a review. J Pharmacol Toxicol Res 2012;5:1-22.
13. PyMol. The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC; 2015.
14. Marvin View (version 18.10.0) Calculation Module Developed by ChemAxon; 2014.
15. National Center for Biotechnology Information. PubChem Compound Summary for CID 5280443, Apigenin. Bethesda, Maryland: National Center for Biotechnology Information; 2021.
16. National Center for Biotechnology Information. PubChem Compound Summary for CID 5281607, Chrysin. Bethesda, Maryland: National Center for Biotechnology Information; 2021.
17. National Center for Biotechnology Information. PubChem Compound Summary for CID 5280445, Luteolin. Bethesda, Maryland: National Center for Biotechnology Information; 2021.
18. Sanner MF. Python: A programming language for software integration and development. J Mol Graph Model 1999;17:57-61.
19. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:42717.
20. Daina A, Zoete V. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem 2016;11:1117-21.
21. Discovery Studio-BIOVIA. Dassault Systèmes, [21.1.0]. [BIOVIA Discovery Studio 2021 Client], San Diego: Dassault Systèmes; 2021.