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Anti-biofilm Activity of Monolaurin on *Staphylococcus aureus*: An *in-silico* Study

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ABSTRACT

Background: *Staphylococcus aureus* is a gram-negative bacterium that can build strong biofilms on biotic and abiotic surfaces, quickly acquire drug resistance mechanisms, and cause major issues with the treatment of hospital infections. The creation of new therapeutic options has become important due to the limited supply of new antibacterial medications. One of the main sources of bioactive molecules is medicinal plants, and monolaurin is a naturally occurring substance with a variety of biological functions. In light of this, the goal of this study was to assess monolaurin's antibiofilm activity against *S. aureus*.

Methods: Using the AutoDock programme, a docking study of monolaurin against Clf A (clumping factor A) was carried out, and Pymol software was used to evaluate the generated hydrogen bonds in the docked complex. This study demonstrates the positive potential of monolaurin as an antibacterial product and lends support to upcoming pharmacological research on this molecule with an eye toward its therapeutic use.

Results: Research was done to support the theoretical absorption of monolaurin in this work and *in silico*. It was feasible to forecast if the monolaurin molecule may be produced as a medication based on the values of the physical-chemical parameters evaluated using the online tool Swiss ADME.

Conclusion: The compound monolaurin demonstrated good receptor ClfA binding affinity with an estimated binding energy of kcal/mol. Natural anti-staphylococcal chemical monolaurin was used as a possible medicine for treating staphylococcal infections in humans by carrying out drug design studies for *S. aureus*.

Key-words: Biofilm, Binding energy, Infection and Immunity, Ligand and Docking, Monolaurin

INTRODUCTION

Staphylococcus aureus has a high prevalence of morbidity and mortality, which results in a wide range of infections and considerable costs for healthcare systems [1].

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S. aureus is naturally resistant to almost all antibiotics that have already been created, but it is also capable of quickly acquiring drug resistance mechanisms, which makes it difficult to utilize even the strongest antibacterial agents, like methicillin and vancomycin [2]. There haven't been many new antibacterial medications introduced in recent years, despite the rise in bacterial antibiotic resistance. This indicates that there is a significant unmet medical demand for novel antibiotics [3]. Bacterial biofilms are common and are thought to be highly structured collections of cells that can attach to surfaces and produce an extracellular matrix [4]. Many

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clinically significant pathogenic bacteria are known to generate biofilms, with *S. aureus, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa* being notable examples [5]. Numerous hospitals acquired infections have been linked to biofilms. Healthcare costs have increased significantly as a result of the chronic tissue- and device-associated infections caused by *S. aureus* biofilm and their innate resistance to the effects of treatment antibiotics [6]. It is vital to provide novel therapeutic options due to the ongoing evolution of drug-resistant bacteria and the scarcity of medicines with antibiofilm action [7]. In this regard, one of the primary sources of bioactive compounds for antibacterial uses is medicinal plants [8]. Natural remedies can be quite effective at destroying bacterial biofilms of many kinds. In addition to operating against strains resistant to traditional medications, such compounds typically have reduced harmful effects and can operate as a template for numerous chemical alterations intended to increase their efficacy [9].

Glycerol monolaurate, or monolaurin, is a surfactant that was initially made accessible as a dietary supplement in 1960 [10]. Monolaurin is prized for its health advantages, particularly as an immune system booster. Monolaurin has been studied as a dietary supplement for its potential to strengthen the immune system against a variety of illnesses, including the common cold, influenza, swine flu, herpes simplex, and other chronic metabolic inflammatory disorders [11,12]. Monolaurin is largely produced chemically from glycerol and lauric acid as an ester (monoester) (12-carbon medium-chain saturated fatty acid). Coconut oil is the richest source of monolaurin, a naturally occurring monoester [13]. Human breast milk contains a sizable amount of GML as well [14]. Palm kernel oil is another source of GML and is significantly more susceptible to enzymatic degradation via hydrolysis. GML is a prime contender for the cosmetic sector, where it is employed as a surfactant, a dispersant, and an emulsifier agent, in addition to serving as an immune booster and nutritional supplement [15]. In the past few decades, the usage of GML as a food ingredient, emulsifier, and preservative has expanded significantly. The antibacterial characteristics of GML are well known, however, un-saturation, the length of the carbon backbone, and cis/trans orientation change the activity [16]. According to reports, GML exhibits antibacterial activity that is more than 200 times greater than that of lauric acid [17].

Numerous studies have shown that GML inhibits the growth of a variety of microbial species, including *S. aureus, Enterococcus faecalis, P. aeruginosa*, and *Acinetobacter baumannii.* Additionally, GML has shown antiviral activity against a variety of RNA-positive strand viruses, including influenza and coronavirus [18]. Although the antiviral potential of GML is not fully understood, preliminary research has shown that GML nutritional supplements enhance host immunity and control interferon pathways [19]. The ability of GML to modify interferon and other antiviral pathways makes it effective against the zika, mumps, and yellow fever viruses. *Ex-vivo* experiments have concurrently shown that GML suppresses the production of pro-inflammatory cytokines [20]. A wise strategy for reducing the threat of infections caused by *S. aureus* bio-films could be the creation of small molecule ligands that precisely target crucial stages involved in biofilm production. In this study, natural anti-staphylococcal chemicals were designed into drugs, and the AutoDock tool was used to dock monolaurin against ClfA (clumping factor A).

MATERIALS AND METHODS

The study was carried out in 2022 (July to September) using a high computing configuration system with RAM 8 gb and Linux operating system with a processor speed 2.4 ghz. The study was carried out using AutoDock Vina 1_1_2 version (https://vina.scripps.edu/). Other tools were used in the study as free versions from ExPasy tools.

Target and Ligand identification- Several publications were used to identify the ligand and target. It was discovered that the substance monolaurin has anti-biofilm activity and that Staphylococcus aureus clumping factor A is one of the crucial proteins involved in the biofilm development process. Therefore, these compounds were taken for additional examination.

Lipinski rule analysis using the Swiss ADME- Swiss ADME technique was used to examine the monolaurin's drug-likeness characteristics. Input for it came in the form of compound grins. The Swiss ADME server was used to forecast the molecular characteristics and biological activity of the medicines displaying strong affinity.

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Docking

Target preparation- The three-dimensional structure of ClfA (PDB ID: 1N67) was retrieved from Research Collaborator for Structural Bioinformatics (RCSB) Protein data bank.

Ligand preparation- The PubChem database was used to acquire the monolaurin structure. The Open Babel converter tool is then used to convert the structure that was obtained from PubChem to PDB files.

Ligplot analysis using PDB sum- LIGPLOT v.4.5.3 PDB sum program was used to predict the binding sites of the drug monolaurin in a receptor ClfA. Four letter pdb code of ClfA (1N67) was given as an input. From input Protein Data Bank files, the LIGPLOT application mechanically creates schematic 2-D representations of protein-ligand interactions. The LIGPLOT diagram showed a schematic representation of all hydrogen bonds and non-bonded contacts between the residues of the protein molecules in the structure and the ligand. Hydrogen bonds were displayed as green dashed lines that were identified with the bond's length in degrees (A°).

Docking using AutoDock- Using AutoDock vina, the ligand analogue monolaurin was docked to the ClfA receptor. AutoDock forecasts how ligands and targets will interact, and it frequently ranks molecules with more subtle affinities. The initial step is to retrieve the target (ClfA.pdb) and ligand (monolaurin chemical compound.pdb) files from databases. The preparation of grid and parameter files (config.txt) for AutoDock Vina, ClfA. pdbqt, and monolaurin chemical molecule in PDBQT format is the second stage. A docked complex was produced in the end.

Analysis of docked complex using Pymol- Using the offline structure visualization programme Pymol, the hydrogen bond formation between the ligand and receptor in the docked complex was examined. PyMOL is modelling and visualization software for working computational scientists.

RESULTS

Lipinski rules analysis using Swiss ADME- A study was conducted to confirm the theoretical absorption of monolaurin in this work and in Silico. It was feasible to determine whether the monolaurin molecule might be developed as a medicine based on the values of the physical-chemical characteristics shown in Table 1 that were calculated using the online application Swiss ADME. This was done using the Lipinski, Ghose, Veber, and Egan guidelines. Table 1 displays the data that was obtained.

Table 1: In-silico studies of Lipinski's parameters of monolaurin

Parameters	Monolaurin
Physicochemical Properties	
Formula	C15H30O4
Molecular Weight	274.40 g/mol
Num. Heavy atoms	19
Fraction Csp3	0.93
Num. Rotatable Bonds	14
Num. H-bonds acceptors	4
Num. H-bonds donors	2
Molar Refractivity	77.83
TPSA [1]	66.76 Å [2]
Lipophilicity	
Consensus [2] Log $P_{o/w}$ [3]	3.22
Water Solubility	
Log S (Ali)	−5.27
Class [4]	Moderately Soluble
Drug likeness	
Lipinski [5]	Yes; 0 violation
Ghose [6]	Yes
Veber [7]	No; 1 violation: Rotors>10
Egan [8]	Yes
Bioavailability Score	0.55

[1]TPSA=Topological Polar Surface Area; [2]Consensus Log Po/w=Average of all five predictions; [3]Log Po/w=Partition coefficient between n-octanol/water; [4]Class=Ali classes: insoluble<−10<poor<−6<moderately soluble<−4<soluble<−2<very soluble<0<highly; [5]Lipinski=MM≤500; Log Po/w≤5; H-bond donors≤5; H-bond acceptors≤10; [6]Ghose=180≤MM≤480; 20≤No. of atoms≤70; 40≤Molar Refractivity≤130; −0.4≤Log Po/w ≤ 5.6; [7]Veber=Num. Rotatable Bonds≤10; TPSA≤140 Å2; [8]Egan=Log Po/w≤5.88; TPSA≤131.6 Å2

A substance's molecular mass is a crucial metric since a substance's volume and the ease with which a molecule can enter the intracellular environment are both impacted by molecular mass. Monolaurin has a molecular mass of 274.40 g/mol, which is consistent with

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Lipinski's rule. Log P, which is associated with a substance's hydrophobicity and capacity to cross plasma membranes, is another significant parameter. With values for acceptors of 4 and donors of 2, the results shown in Fig. 1 for the number of hydrogen acceptors and donors follow Lipinski's rule. Lipinski's rule of five comparisons of these numbers indicates that monolaurin has excellent theoretical oral bioavailability.

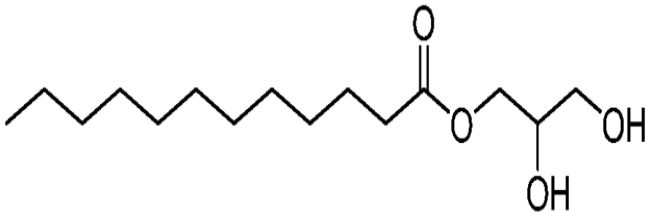

Fig. 1: Structures of monolaurin

LIGPLOT analysis using PDB sum- Total three binding residues Leu 283(A), Lys 281(A) and Asp 340(A) were predicted using PDB sum LIGPLOT analysis, as shown in Fig. 2.

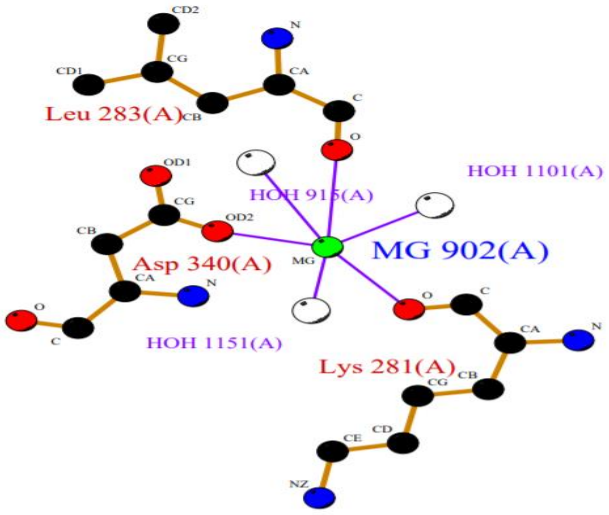

Fig. 2: Ligplot of ClfA

With estimated binding energy of kcal/mol, the molecule monolaurin has demonstrated an excellent affinity for the receptor ClfA. Natural anti-staphylococcal chemical monolaurin will be used as a possible medicine for treating staphylococcal infections in humans by carrying out drug design studies for *S. aureus*.

Docking using AutoDock- The compound monolaurin was docked at the binding sites of ClfA (PDB id:1N67) using the AutoDock tool, which resulted in energy-based descriptors like energy, internal energy, torsional energy, internal energy measure of substance analogue monolaurin against receptor ClfA was performed with estimated binding energy -6.0 kcal/mol.

Hydrogen bond formation analysis of docked complex using Pymol- total 3 hydrogen bonds with Tyr 376, Asp 370 and Asp 340 were analyzed in the monolaurin - ClfA docked complex as shown in Fig. 3.

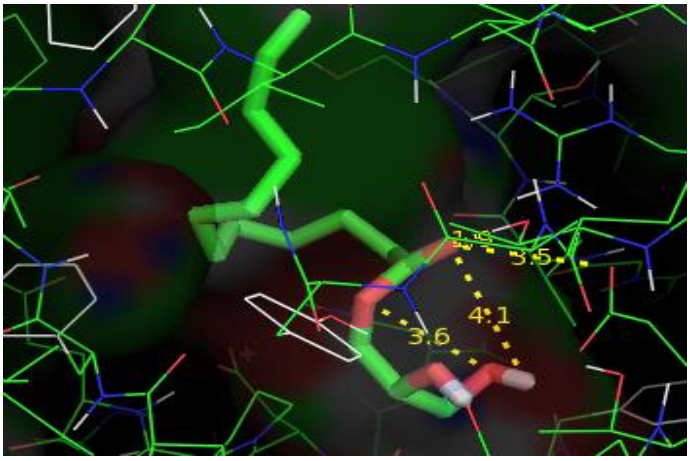

Fig. 3: Pymol analysis of monolaurin-ClfA docked complex

DISCUSSION

The primary threat to human health and the healthcare system is bacterial infections. Several bacterial species have recently developed resistance to a wide variety of antibiotics and antimicrobial agents [19]. In this context, plasmid, transposon, and chromosomal DNA are examples of new processes in that bacteria have evolved [21]. Such a sophisticated strategy increases pathogen pathogenicity [14]. As a result of developed resistance to a variety of antibiotics and antimicrobial medicines, new molecules are needed to combat such infections. A promising nutritional supplement called monolaurin strongly inhibits the production of biofilms by a variety of microorganisms, primarily bacterial species. Additionally, bacteria that lack biofilms or produce them inefficiently are more vulnerable to antibiotics [22]. A study of the clinical use of monolaurin by Archer *et al.* in 2011 led to the conclusion that it had antibacterial properties [23]. According to the study, monolaurin's antimicrobial effectiveness against *S. aureus, Streptococcus pyogenes, S. agalactiae, S. suis, Enterococcus faecalis, Bacillus anthracis Sterne, Campylobacter jejuni*, *Fusobacterium* species, *Pseudomonas* sp. The study also shows that bacteria like *Shigella sonnei, Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella minnesota, E. coli,* and *K. aerogenes* are resistant to monolaurin. *Candida albicans*

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has been shown to have anti-biofilm formation activity in a study by Lopes *et al.* [24].

CONCLUSIONS

Drug development is required for Staphylococcus aureus infections in humans because this bacterium is responsible for many serious and fatal human diseases and has been discovered to be resistant to several antibiotics now on the market. With estimated binding energy of kcal/mol, the molecule monolaurin has demonstrated an excellent affinity for the receptor ClfA. Natural anti-staphylococcal chemical monolaurin will be used as a possible medicine for treating staphylococcal infections in humans by carrying out drug design studies for *S. aureus*.

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