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Analysis Virulence Factor of *Staphylococcus Epidermidis*and*Streptococcus Pyogenes*Targeted by Epigallocatechin Gallate (EGCG) Using A Bioinformatic Approach

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Abstract
Background*: Staphylococcus epidermidis and Streptococcus pyogenes are microorganisms that can cause infectious diseases. The general management for bacterial infections is by using antibiotic therapy. The ease of access to antibiotics means that not all antibiotic users are under the observation of health professionals, which can trigger antibiotic resistance. Epigallocatechin gallate (EGCG) is a natural chemical compound that has been proven in vitro to act actively as an antibacterial agent. However, the antipathogenic of EGCG's abilities to inhibit the virulence factors of Staphylococcus epidermidis and Streptococcus pyogenes are poorly studied. A bioinformatics approach was used to analyze the virulence factors of Staphylococcus epidermidis and Streptococcus pyogenes targeted by EGCG.*
Materials and Methods*: This study was bioinformatics research with samples in the form of FASTA sequences of Staphylococcus epidermidis and Streptococcus pyogenes obtained through the National Center for Biotechnology Information (NCBI). The Software used in the research namely STITCH v5.0, VICMPred, VirulentPred, BepiPred v2.0, and PSORTb v3.0.*
Results*: The results showed there was an interaction between proteins from the two bacteria and Epigallocatechin gallate (EGCG). It was observed that specific proteins possessed virulent characteristics. Moreover, the biological profiles of these proteins were also determined.*
Conclusion:*EGCG effectively affects virulence factors by interacting with several proteins from Staphylococcus epidermidis and Streptococcus pyogenes, as demonstrated through bioinformatics analysis.*
Key Word*:Staphylococcus epidermidis; Streptococcus pyogenes; Epigallocatechin gallate (EGCG); Virulence factors; Bioinformatics.*

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I. Introduction

Infectious diseases pose a significant health issue in communities, particularly in tropical regions. The high humidity levels can contribute to the accelerated development of infectious diseases.[1] Infectious diseases can be caused by various microorganisms, including bacteria, viruses, fungi, and parasites.[2] Some of the bacteria responsible for infectious diseases include *Staphylococcus epidermidis* and *Streptococcus pyogenes*.

Staphylococcus epidermidis and *Streptococcus pyogenes* are Gram-positive bacteria that are part of the normal flora and can be found on the skin, digestive tract, and even in the environment around us. However, under certain conditions, such as a weakened immune system, they can become pathogens that cause infectious skin diseases.[3] The Global Burden of Disease (GBD) recorded an average of 146.84 million cases of bacterial skin diseases globally in 2019, with Southeast Asia having an average of 55.44 million cases.[4]The common treatment method employed to combat bacterial infections is through the administration of antibiotics.[5]

Antibiotics are synthetic or natural compounds that can inhibit biochemical processes during bacterial infections. The ease of public access to antibiotics results in not all antibiotic users being under the supervision of healthcare professionals, a situation that can trigger the development of resistance.[7] Antibiotic resistance can lead to various problems, such as increased morbidity and mortality rates.[8] Therefore, there is a need for alternative therapies, such as the use of natural substances with minimal negative side effects and antibacterial properties. The use of natural substances may be safer for long-term use in the body. Epigallocatechin gallate

(EGCG) is a natural chemical compound that has been proven in vitro to function as an antibacterial agent. However, the validation of this compound's ability to inhibit the virulence factors of *Staphylococcus epidermidis* and *Streptococcus pyogenes* is not yet fully understood. One method that can be employed to analyze the effects of EGCG on the virulence factors of these bacteria is a bioinformatics approach. Therefore, this research aims to analyze the virulence factors of *Staphylococcus epidermidis* and *Streptococcus pyogenes* targeted by EGCG using a bioinformatics approach.

II. Material And Methods

This study was bioinformatics research using FASTA samples of *Staphylococcus epidermidis* and *Streptococcus pyogenes* obtained from the National Center for Biotechnology Information (NCBI). The study aimed to analyzevirulence factors of*Staphylococcus epidermidis* and *Streptococcus pyogenes*targeted by EGCG. The analysis of protein-compound interactions was done by STITCH v5.0. The proteins of *Staphylococcus epidermidis* and *Streptococcus pyogenes* targeted by EGCG were then analyzed for their functional class usingVICMPred.The analysisof the virulence property of the proteins was done using the VirulentPred. BepiPred v.2, MHC I Binding Predictions, and MHC II Binding Predictionswere employed for epitope B cell and T cell analysis. In addition, PSORTb v.3 was employed for subcellular protein localization analysis.

III. Result and Discussion

Protein Interaction Analysis

This analysis was conducted using STITCH v5.0. From the analysis, several proteins from *Staphylococcus epidermidis* and *Streptococcus pyogenes* were found to interact with EGCG. Additionally, several proteins were identified to interact with each other.

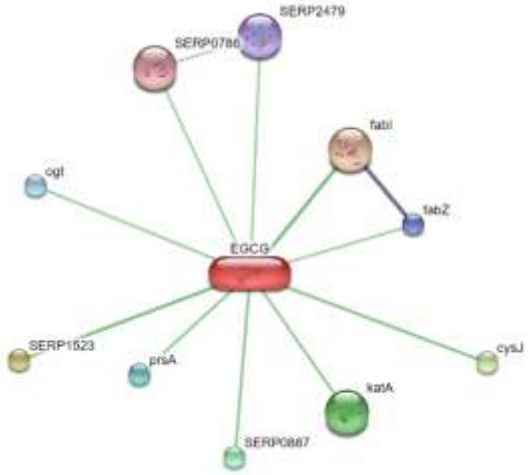

Figure 1. Results of the Interaction Analysis of *Staphylococcus epidermidis* with EGCG; ten interacting proteins were identified, namely SERP2479, fabl, fabZ, cysJ, katA, SERP0887, prsA, SERP1523, ogt, SERP0786.

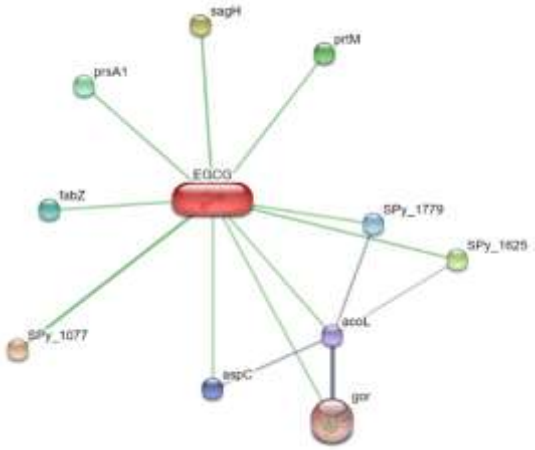

Figure 2. Results of the Interaction Analysis of *Streptococcus pyogenes* with EGCG; ten interacting proteins were identified, namely sagH, prtM, Spy_1779, Spy_1625, acoL, gor, aspC, Spy_1077, fabZ, and prsA1.

Functional Class and Virulence Property Analysis of Proteins

This analysis represents a subsequent stage following the protein interaction analysis. VICMPred and VirulentPred were used for the analysis, yielding results in terms of functional classes and virulence properties for each protein, along with their virulence scores.

Table 1. Results of Functional Class and Virulence Property Analysis of *Staphylococcus epidermidis* Proteins Interacting with EGCG.

Organism	Identifier	Protein Which Interacts With EGCG	VICMPred Functional Class	VirulentPred	VirulentPred Score
Staphylococcus epidermidis	SERP2479	Protein kinase domain-containing protein	Cellular process	Virulent	0.67
	fabI	enoyl-ACP reductase	Cellular process	Non-Virulent	-0.35
	fabZ	(3R)-hydroxymyristoyl-ACP dehydratase	Metabolism Molecule	Non-Virulent	-1.00
	cysJ	sulfite reductase (NADPH) flavoprotein alpha-component	Cellular process	Non-Virulent	-1.01
	katA	catalase	Cellular process	Non-Virulent	-1.02
	SERP0887	ABC transporter permease	Metabolism Molecule	Virulent	0.53
	prsA	protein export protein PrsA	Cellular process	Non-Virulent	-0.69
	SERP1523	DNA-cytosine methyltransferase	Cellular process	Non-Virulent	-0.07
	ogt	methylated-DNA--protein-cysteine methyltransferase	Cellular process	Non-Virulent	-0.83
	SERP0786	serine/threonine protein kinase	Metabolism Molecule	Non-Virulent	-0.11

Table 2. Results of Functional Class and Virulence Property Analysis of *Streptococcus pyogenes* Proteins Interacting with EGCG.

Organism	Identifier	Protein Which Interacts With EGCG	VICMPred Functional Class	VirulentPred	VirulentPred Score
Streptococcus pyogenes	sagH	ABC transporter ATP-binding protein	Metabolism Molecule	Non-Virulent	-0.31
	prtM	foldasePrsA	Metabolism Molecule	Non-Virulent	-0.67
	SPy_1779	aminotransferase	Metabolism Molecule	Non-Virulent	-0.97
	SPy_1625	protein kinase	Metabolism Molecule	Virulent	0.82
	acoL	dihydrolipoamide dehydrogenase, component E3	Virulence factors	Non-Virulent	-1.01

	gor	glutathione reductase	Metabolism Molecule	Non-Virulent	-1.03
	aspC	aspartate aminotransferase	Cellular process	Non-Virulent	-1.02
	SPy_1077	methyl transferase	Cellular process	Non-Virulent	-0.18
	fabZ	(3R)-hydroxymyristoyl-ACP dehydratase	Metabolism Molecule	Non-Virulent	-0.99
	prsA1	Foldase protein PrsA	Cellular process	Virulent	0.21

B-cell Epitope Analysis

This analysis used BepiPred v2.0, focusing on virulent proteins, namely SERP2479 and SERP0887 in *Staphylococcus epidermidis*, as well as Spy_1625 and prsA1 in *Streptococcus pyogenes*.

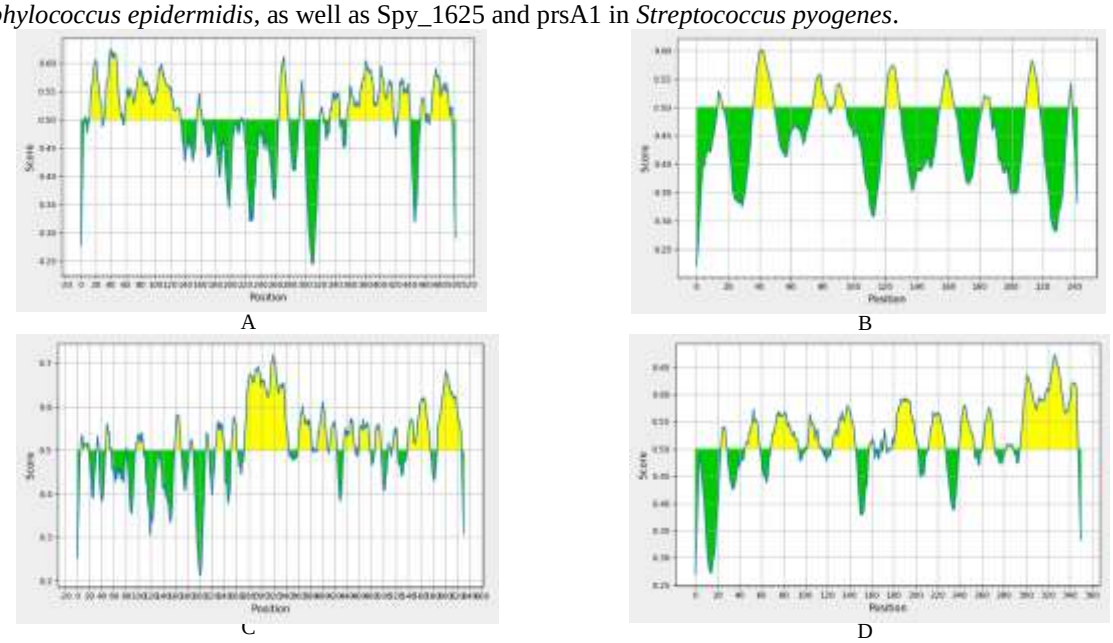

Figure 3. Results of B-cell Epitope Analysis on (A) Protein kinase domain-containing protein (SERP2479), (B) ABC transporter permease (SERP0887), (C) Protein kinase (Spy_1625), and (D) Foldase protein PrsA (prsA1)

T-cell Epitope Analysis

This analysis used MHC I Binding Predictions and MHC II Binding Predictions, focusing on virulent proteins. The analysis revealed several peptide chains capable of binding to T cells (epitopes) along with their corresponding values and rankings.

Table 3. Results of MHC I Analysis for SERP2479 Interacting with EGCG

Allele	**Start**	**End**	**Length**	**Peptide**	**Score**	**Percentile Rank**
HLA-A*11:01	257	265	9	KISDLGLGK	0.8789	0.03
HLA-A*11:01	145	153	9	KSTGLAVKK	0.8178	0.07
HLA-A*11:01	333	341	9	ATNSSKEYR	0.7286	0.12
HLA-A*11:01	319	327	9	VVNNHHLFR	0.7088	0.13
HLA-A*11:01	359	367	9	HSSAKHVEK	0.6444	0.18

Table 4. Results of MHC I Analysis for SERP0887 Interacting with EGCG

Allele	**Start**	**End**	**Length**	**Peptide**	**Score**	**Percentile Rank**
HLA-A*11:01	3	11	9	STYFKIELK	0.9673	0.01

HLA-A*11:01	91	99	9	SSFQYYLVK	0.9004	0.02
HLA-A*11:01	231	239	9	SIALFMNKK	0.8104	0.07
HLA-A*11:01	230	238	9	LSIALFMNK	0.6248	0.19
HLA-A*11:01	113	121	9	IIFSVAHFY	0.5050	0.29

Table 5. Results of MHC I Analysis for Spy_1625 Interacting with EGCG

Allele	Start	End	Length	Peptide	Score	Percentile Rank
HLA-A*11:01	122	130	9	SAMTLAHQK	0.9673	0.02
HLA-A*11:01	329	337	9	ALQPPTKKK	0.8516	0.05
HLA-A*11:01	246	254	9	VVIRATAKK	0.8203	0.06
HLA-A*11:01	219	227	9	TIALQHFQK	0.7854	0.08
HLA-A*11:01	512	520	9	ITMPMVTEY	0.7308	0.12

Table 6. Results of MHC I Analysis for prsA1 Interacting with EGCG

Allele	Start	End	Length	Peptide	Score	Percentile Rank
HLA-A*11:01	103	111	9	SSLTPETFK	0.9218	0.01
HLA-A*11:01	223	231	9	VLDPTSYQK	0.7847	0.08
HLA-A*11:01	75	83	9	KVSKKEVEK	0.7697	0.09
HLA-A*11:01	70	78	9	AQYGDKVSK	0.7610	0.1
HLA-A*11:01	272	280	9	KVIANALDK	0.6748	0.16

Table 7. Results of MHC II Analysis for SERP2479 Interacting with EGCG

Allele	Start	End	Length	Peptide	Score	Percentile Rank
HLA-DRB1*04:01	153	167	15	KLKEEYLTDSSIKSR	0.9750	0.01
HLA-DRB1*04:01	154	168	15	LKEEYLTDSSIKSRF	0.9802	0.01
HLA-DRB1*04:01	155	169	15	KEEYLTDSSIKSRFK	0.9860	0.01
HLA-DRB1*04:01	156	170	15	EEYLTDSSIKSRFKR	0.9551	0.06
HLA-DRB1*04:01	108	122	15	NHYGYYITGTNNARY	0.9382	0.11

Table 8. Results of MHC II Analysis for SERP0887 Interacting with EGCG

Allele	Start	End	Length	Peptide	Score	Percentile Rank
HLA-DRB1*04:01	148	162	15	LGLIIAQLNDIQKAS	0. 3564	5.2
HLA-DRB1*04:01	147	161	15	TLGLIIAQLNDIQKA	0.2779	6.9
HLA-DRB1*04:01	31	45	15	YLLFTSILDMPEEAK	0. 2343	8.2
HLA-DRB1*04:01	198	212	15	TYNLKLLAIDLAQNK	0.2207	8.7
HLA-DRB1*04:01	149	163	15	GLIIAQLNDIQKASS	0.2208	8.7

Table 9. Results of MHC II Analysis for Spy_1625 Interacting with EGCG

Allele	Start	End	Length	Peptide	Score	Percentile Rank
HLA-DRB1*04:01	549	563	15	ATGFVPIHSPSSKAI	0.9712	0.01
HLA-DRB1*04:01	548	562	15	SATGFVPIHSPSSKA	0.9732	0.01
HLA-DRB1*04:01	45	59	15	RTNYQTDQVAVARFQ	0.9642	0.02
HLA-DRB1*04:01	547	561	15	SSATGFVPIHSPSSK	0.9486	0.06
HLA-DRB1*04:01	44	58	15	LRTNYQTDQVAVARF	0.9434	0.09

Table 10. Results of MHC II Analysis for prsA1 Interacting with EGCG

Allele	Start	End	Length	Peptide	Score	Percentile Rank
HLA-DRB1*04:01	189	203	15	KVTYKFDSGATNVPT	0.7753	0.9
HLA-DRB1*04:01	188	202	15	KKVTYKFDSGATNVP	0.7406	1.1
HLA-DRB1*04:01	287	301	15	DKAFANILAQYANLG	0.6508	1.7
HLA-DRB1*04:01	152	166	15	MITLDNEETAKSVLE	0.5954	2.0
HLA-DRB1*04:01	230	244	15	QKKFYIVKVTKKAEK	0.6081	2.0

Subcellular Location Analysis

This analysis used PSORTb v3.0, and the proteins selected for analysis were those with virulent characteristics.

Table 11. Results of Subcellular Location Analysis of Virulent Proteins in *Staphylococcus epidermidis* and *Streptococcus pyogenes*.

Organism	Identifier	Functional Proteins	Subcellular Location
Staphylococcus epidermidis	SERP2479	Protein kinase domain-containing protein	Cytoplasmic membrane
	SERP0887	ABC transporter permease	Cytoplasmic membrane
Streptococcus pyogenes	Py_1625	Protein kinase	Cytoplasmic membrane
	PrsA1	Foldase protein PrsA	Cytoplasmic membrane

IV. Discussion

Based on the results, an analysis of protein-compound interactions was done by STITCH v5.0, it was found that there were ten proteins from *Staphylococcus epidermidis* capable of interacting with EGCG, namely SERP2479, fabI, fabZ, cysJ, katA, SERP0887, prsA, SERP1523, ogt, SERP0786. Additionally, there were ten proteins from *Streptococcus pyogenes*, namely sagH, prtM, Spy_1779, Spy_1625, acoL, gor, aspC, Spy_1077, fabZ, and prsA1 (Figure 1 and Figure 2). These proteins were mentioned in various forms of identification codes (identifier). Furthermore, it was known that some of these proteins also interacted with each other. Interactions between proteins can occur to assist cells in carrying out their biological functions.[8] In bioinformatics, the interactions among proteins targeted by EGCG were identified due to the similarity in their amino acid sequences, and some of them had similar functions. Interactions between proteins that were found in this study occurred due to the similarity in amino acid sequences. The results of interactions using the bioinformatics method also differ from the in vitro method, wherein a study conducted in vitro by Azizah et al. (2020), it was found that green tea ethanol extract, whose main component is EGCG, has antibacterial activity by inhibiting

bacterial growth.[9] However, the specific part of the bacteria that interacts with EGCG, leading to its growth inhibition, is unknown. On the other hand, this study identified the specific proteins that interact with EGCG.

Most of the interacting proteins play crucial roles in the survival of *Staphylococcus epidermidis* and Stre*ptococcus pyogenes*. Based on the results of functional class and virulence property analysis using VICMPred, it was found that the majority of proteins from *Staphylococcus epidermidis* and *Streptococcus pyogenes*capable of interacting with EGCG have functions related to cellular processes and metabolism of molecules (Table 1 and Table 2). The term "cellular process" refers to cell division, membrane biogenesis, cell movement, and signal transduction molecules, while "metabolism molecule" includes energy production, transportation, and the metabolism of carbohydrates, amino acids, nucleotides, and lipids.[10]

Proteins in *Staphylococcus epidermidis* with cellular process functions include Protein kinase domain-containing protein, enoyl-ACP reductase, sulfite reductase (NADPH) flavoprotein alpha-component, catalase protein export protein PrsA, DNA-cytosine methyltransferase, and methylated-DNA--protein-cysteine methyltransferase. Meanwhile, those with metabolism molecule functions include (3R)-hydroxymyristoyl-ACP dehydratase, ABC transporter permease, and serine/threonine protein kinase. In *Streptococcus pyogenes*, proteins with cellular process functions include aspartate aminotransferase, methyl transferase, and Foldase protein PrsA. Those with metabolism molecule functions include ABC transporter ATP-binding protein, foldasePrsA, aminotransferase, protein kinase, glutathione reductase, and (3R)-hydroxymyristoyl-ACP dehydratase. Additionally, it was known that the protein dihydrolipoamide dehydrogenase/component E3 with the identifier acoL functioned as a virulence factor. Virulence factors help bacteria attack the host, cause diseases, and evade host defenses. Some types of virulence factors include adhesion factors, invasion factors, capsules, endotoxins, exotoxins, and siderophores.[11] However, according to VICMPred, the classification of a protein as a virulence factor is based on adhesion factors, toxins, and hemolytic molecules.[10] The Analysis using VICMPred was useful in identifying the functional properties of each of these bacterial proteins.

Further analysis was conducted using VirulentPred to determine the virulence properties of each protein from*Staphylococcus epidermidis* and *Streptococcus pyogenes*capable of binding with EGCG. From this analysis, it is revealed that out of the ten proteins from*Staphylococcus epidermidis*, two possess virulent properties, namely SERP2479 and SERP0887, with virulence scores of 0.67 and 0.53, respectively (Table 1). Similarly, from the ten proteins of *Streptococcus pyogenes*, two had virulent properties, namely Spy_1625 and prsA1, with virulence scores of 0.82 and 0.21, respectively. (Table 2). Bacterial virulence can be interpreted in terms of its relative ability to cause disease. It is usually measured by the number of bacteria causing infection, the route of entry of bacteria into the host's body, and bacterial virulence factors.[12] Virulence factors can be encoded on the chromosome, plasmid, transposon, or DNA bacteriophage.[11]

The Protein Kinase Domain-Containing Protein (SERP2479) in *Staphylococcus epidermidis* and the Protein Kinase (SPy_1625) in *Streptococcus pyogenes*, despite having different functional classes, share some similar roles in their virulent properties. Both proteins, while playing crucial roles in the survival of their respective bacteria, also contribute significantly to their virulence. The kinase in both proteins can interact with host substrates, disrupting essential functions of the host, such as the host's immune response, cell morphology, and host integrity.Bacteria-secreted kinase will phosphorylate host substrates, disrupting the nuclear factor kappa-B (NFKB) pathway, which is a protein that functions as a transcription factor, especially for genes involved in immune/inflammatory responses.[13]The ABC transporter permease protein (SERP0887) in *Staphylococcus epidermidis* not only plays a crucial role in the bacteria's survival by participating in molecular metabolism but is also significantly involved in bacterial pathogenesis and virulence. SERP0887 is essential in acquiring and transporting various nutrients required by the bacteria, such as iron ions, zinc, magnesium, amino acids, and choline compounds.These nutrients, through bacterial metabolic mechanisms and transporter proteins, become crucial determinants of virulence, contributing to the bacteria's ability to cause diseases in the host.[14]

The foldase protein PrsA (prsA1) is a protein commonly found in Gram-positive bacteria, such as *Streptococcus pyogenes*. prsA1 not only played a crucial role in the bacteria's survival by participating in cellular processes but also contributed to infecting its host. prsA1 was involved in the maturation process of SpeB, a cysteine protease crucial for virulence factors. Furthermore, prsA1 played a significant role in protein secretion by assisting in extracellular folding post-translocation of several secreted proteinsAdditionally, the amount of prsA1 secretion could influence biofilm formation, where biofilm is one of the virulence factors. The deficiency of prsA1 could significantly decrease the production of insoluble glucans, resulting in reduced sucrose adhesion and biofilm formation.[15]

The functional class and virulence property analysis results on *Streptococcus pyogenes* proteins reveal that dihydrolipoamide dehydrogenase/component E3 (acoL), based on functional class analysis, had a function as a virulence factor. However, according to the virulence property analysis, this protein had no virulent properties (Non-virulent). This discrepancy arose because the virulence property analysis indicated that the protein had a score of -1.01, which was negative, implying that the score for acoL was insufficient to confer virulence to the protein.

The virulent proteins of *Staphylococcus epidermidis* and *Streptococcus pyogenes* underwent an analysis of B-cell epitope locations using BepiPred v2.0. BepiPred has the highest prediction accuracy on test datasets and has proven to outperform all other tested methods on validation datasets.[16] The B-cell epitope analysis provides specific locations on bacterial proteins capable of binding to antibodies or the immune system identified through bioinformatics. Antibodies are crucial components of the immune system in all vertebrates. They can identify and neutralize foreign entities that can stimulate the immune system, such as viruses, bacteria, fungi, cancer cells, and some toxins, by binding to specific regions on their surface, commonly known as antigens. Specific parts of an antibody bind to specific regions on the antigen, referred to as epitopes. The B-cell epitope analysis focuses on selecting the strongest epitopes that can serve as potential targets for diagnostic and epitope-based vaccine production.[17]The results of this analysis revealed that there were specific amino acid sequences within each protein capable of binding to B cells (epitopes). Furthermore, the analysis clarified that there were molecular interactions between *Staphylococcus epidermidis* and *Streptococcus pyogenes* proteins and EGCG, manifested as B-cell binding marked by epitopes shown in yellow in graphical representations (Figure 3 - Figure 6). The binding of antibacterial active compounds like EGCG to these specific locations triggers an immune response to produce antibodies.[18] The binding or attachment of EGCG to epitopes also prevents the adhesion of pathogenic bacteria to the host cell membrane, where EGCG disrupts the structure, such as bacterial peptidoglycan.[19]

The results of the B-cell epitope analysis in this study align with previous research conducted by Rian KP (2023), who using similar analysis methods and compounds, stated that the identification of numerous peptide epitopes supports the use of EGCG as a phytochemical compound with antibacterial activity against P. *acnes*.[20]

The virulent proteins of *Staphylococcus epidermidis* and *Streptococcus pyogenes* underwent analysis for MHC I and MHC II using MHC I Binding Predictions and MHC II Binding Predictions. The MHC I analysis focused on peptide chains capable of binding to cytotoxic T cells, revealing several peptide chains with the ability to bind to cytotoxic T cells, along with their corresponding scores and rankings (Table 5.3 - Table 5.6). The binding of peptides to MHC I molecules forms a closed groove composed of a single α chain. The peptide binding pathway of MHC I also includes binding with a strict phytochemical preference. These factors contribute to the analysis results, indicating consistently short peptide chain lengths, around 9 amino acids.[21]The choice of HLA-A11:01 in MHC I analysis is based on previous research by Habel JR et al. (2022), where the study indicated the highest detection of HLA-A11:01 in the Asian population, particularly in Southeast Asia. Therefore, the use of HLA-A*11:01 is expected to represent this population.[22]

Previous research conducted by Simarmata et al. (2022), indicated that among the T-cell epitopes produced, the peptide ATEDPSSGY with a score of 2.80 is a candidate peptide for an Ebola vaccine due to its highest value. This suggests that the MHC I Analysis can have significant implications in the development of T-cell-based immunotherapy. T-cell epitopes bind to MHC to trigger an immune response. T cells can elicit a specific adaptive immune response against pathogens. The recognition of epitopes by T cells and the induction of immune responses play a key role in an individual's immune system.[23] The scores and percentile rank in the analysis results provide an overview of how well an epitope can bind to a specific MHC I allele and the extent to which the epitope can be found in the population of amino acid sequences.[24]

The MHC II analysis focused on peptide chains capable of binding to helper T cells. From this analysis, peptide chains that can bind to helper T cells were identified, complete with their scores and rankings (Table 7 – Table10). In MHC II, the peptide binding pathway is open, resulting in a more varied peptide chain length ranging from 9 to 22 amino acids.[21] These results indicate that the body's immune system has the potential to recognize and respond effectively to bacterial proteins, which could have important implications in the development of T cell-based immunotherapy. Additionally, it provides crucial insights into vaccine development by allowing researchers to identify specific parts of pathogens that are likely to stimulate a strong immune response.[23]

The subcellular localization analysis of virulent proteins in *Staphylococcus epidermidis* and *Streptococcus pyogenes* using PSORTB v.3.0 revealed that the subcellular location of virulent proteins in both *Staphylococcus epidermidis* and *Streptococcus pyogenes* is situated on the cytoplasmic membrane. The cytoplasmic membrane, also known as the plasma membrane, is located on the inner part of the cell wall and is formed by lipoproteins and proteins, including penicillin-binding proteins. The plasma membrane plays a role in cell wall synthesis, essential in pathogenesis through endostreptosin, acts as a selectively permeable barrier or a highly selective gatekeeper determining which components are allowed to enter and exit the cell, facilitates active transport, and controls the internal composition of bacterial cells.[25]

The protein kinase domain-containing protein (SERP2479) in *Staphylococcus epidermidis* and the protein kinase (SPy_1625) in *Streptococcus pyogenes* has the same subcellular location, specifically on the cytoplasmic membrane. Protein kinase is a crucial component in cellular signaling pathways as it plays a role in intracellular transduction to regulate the growth and proliferation of many proteins and can trigger and regulate

immune responses. This is possible because kinases can transfer high-energy phosphate donors to other proteins, where phosphate is transferred from ATP to serine, threonine, or tyrosine residues in proteins by protein kinases. Most cellular pathways, especially those involved in signal transduction, are generally regulated by kinases.[26] Kinases translocated from the cytoplasm to the cytoplasmic membrane play a crucial role in signaling cascades, ranging from cell adhesion, cell growth, proliferation, and differentiation to cell death.[27] Kinases are essential in the initial stages of intracellular immune cell signaling. Kinases can bind to intracellular receptors on the surface of T and B cells, and upon binding, they trigger intracellular signaling cascades within the cell.[26]

The ABC transporter permease protein (SERP0887) in *Staphylococcus epidermidis* was identified to be located in the cytoplasmic membrane. Its subcellular location is associated with its active role in translocating various substrates. This protein has dual functions, serving as both an importer and an exporter. The exporter ABC transporter permease can translocate various molecules, such as proteins, toxins, and xenobiotics, out of the cytoplasm. Exporter ABC transporter permease plays a crucial role in bacteria's physiology, pathogenesis, and virulence by exporting proteins like toxins, surface layer proteins, and polysaccharides. On the other hand, the importer ABC transporter permease facilitates the acquisition and import of various essential compounds, such as metal ions and proteins, from the extracellular matrix into the cell. In bacteria, the role of ABC transporter permease is frequently found as an importer because it can obtain essential nutrients for bacterial survival and pathogenesis. One of the components of the importer ABC transporter permease is the substrate-binding protein, where this substrate-binding protein is also located in the cytoplasmic membrane, either directly binding to the importer or associating with lipoproteins bound to the bacteria. Bacteria leverage the role of ABC transporter permease in acquiring nutrients as a key determinant of virulence to adapt to changes in the host environment rapidly. This adaptation allows bacteria to acquire nutrients at low concentrations during the innate host response, facilitating bacterial colonization and the formation of diseases in the host. The significant role of ABC transporter permease in bacterial virulence lies in transporting nutrients such as ions, amino acids, and proteins.[14] According toUshanthika (2019), which found that EGCG can interact with ABC transporter permease, where this protein plays a crucial role in nutrient and metal ion absorption, thus influencing bacterial survival. Therefore, EGCG can be a promising compound to inhibit infections caused by pathogens.[18]

The subcellular location of the Foldase protein PrsA (prsA1) in *Streptococcus pyogenes* is also in the cytoplasmic membrane. The subcellular location of this protein is closely related to its role in protein secretion, specifically aiding in the extracellular folding post-translocation of several secreted proteins. PrsA1 is part of the parvulin peptidyl-prolyl isomerase (PPIase) that catalyzes the cis-trans isomerization of the N-terminal peptide bond to proline residues in polypeptide chains. PrsA1 is a lipid-modified protein that attaches to the outer membrane layer, but a significant portion is also secreted. PrsA1 plays a crucial role in bacterial growth, and a deficiency in PrsA1 can lead to a decrease in cell wall integrity. Besides its role in protein folding, PrsA1 is also involved in the biogenesis of the cell wall, toxins, and virulence factors. Due to its involvement in modulating pathogenicity factors, PrsA1 becomes a potential target for antimicrobial drugs.[28,29]

V. Conclusion

This study proves that EGCG as a natural chemical compound effectively acts as an antibacterial agent, affecting virulence factors by interacting with several proteins of *Staphylococcus epidermidis* and *Streptococcus pyogenes* through bioinformatics. It is known that virulent proteins in *Staphylococcus epidermidis* and *Streptococcus pyogenes* interacting with EGCG are located in the subcellular membrane, indicating that EGCG's ability to prevent the adhesion of pathogenic bacteria to the host cell membrane by disrupting structures such as bacterial peptidoglycan is highly effective for both bacteria.

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