UNIVERSITY OF CAPE COAST

IN VITRO INHIBITORY EFFECT AND MOLECULAR INTERACTION STUDIES OF THE BIOACTIVE COMPOUNDS IN ETHANOLIC EXTRACTS OF GARLIC, TURMERIC, AND CINNAMON ON AMPICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

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JUNE 2024

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BY

ALBERT ACHIROFI

A THESIS SUBMITTED TO THE DEPARTMENT OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY, OF THE SCHOOL OF BIOLOGICAL SCIENCES, COLLEGE OF AGRICULTURE AND NATURAL SCIENCES, UNIVERSITY OF CAPE COAST, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN MOLECULAR BIOLOGY AND BIOTECHNOLOGY

JUNE, 2024

DECLARATION

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree at this University or elsewhere.

Candidate's Signature……. Date…….

Name:…….

Supervisors Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature…… Date……

Name: ……..

Co-Supervisor's Signature……. Date…….

Name: ……

ABSTRACT

Antimicrobial resistance occurs when microbes stop responding to antibiotics used to treat their infections. This study investigated the inhibitory effect of ethanolic extracts of garlic, cinnamon, turmeric and the combination of each plant extract with ampicillin to help overcome antimicrobial resistance. It also conducted molecular docking analysis of the bioactive compounds in garlic, cinnamon and turmeric to help in drug design to curb antimicrobial resistance. The inhibitory effect was carried out using the agar disc diffusion method whilst Gold software and BIOVIA Discovery Studio was used for the molecular docking studies. The *Staphylococcus aureus* was sensitive to the turmeric extracts, a combination of turmeric and ampicillin but moderately sensitive to the ampicillin. The molecular docking studies of curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingiberene, curcumenol, and curcumol with Sortase A protein predicted the binding efficiency which are evidenced by a fitness score of 65.72%, 63.46%, 62.37%, 61.80%, 49.19%, 41.26%, and 37.47% respectively. The *Escherichia coli* was moderately sensitive to the cinnamon extract and ampicillin but sensitive to the combination of the cinnamon extract and ampicillin, the garlic extract, and a combination of the garlic and ampicillin. Molecular docking studies of eugenol, cinnamyl acetate, linalool, beta-caryophyllene, and cinnamaldehyde with beta-lactamase predicted the binding efficiency which are evidenced by a fitness score of 40.64%, 39.62%, 38.03%, 36.86%, and 35.25% respectively. Thus, the various extract were good inhibitors of the bacteria and molecular docking studies predicted the binding mode and binding efficiency of the ligands.

DEDICATION

To my friend, Kelvin Israel Afful.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Antimicrobial resistance occurs when microbes stop responding to antimicrobials such as antibiotics, antifungal and antiviral, hence allowing infections to be difficult to cure and also multiplying the risk of disease transmission and possible death (Kirui, 2022).

Globally, antimicrobial resistance has caused about 1.27 million deaths and in 2019 only, there were almost 5 million deaths, which serves as a severe threat to public health (Kirui, 2022). According to the Center for Disease Control (2019), antimicrobial resistance is a threat to Africa than HIV/AIDS, tuberculosis, and malaria, with children being at risk. These three diseases now account for fewer death than antimicrobial resistance, which has a death rate of 27.3 deaths per 100,000 people which makes it the highest globally. The Center for Disease Control (2019), has reported that, in 2019, Ghana experienced serious issues with antimicrobial resistance resulting in 5,900 direct deaths and an additional 25,300 related deaths. Ghana was ranked 36th worldwide for age-standardized mortality rates linked to antimicrobial resistance per 100,000 population.

In Ghana, Kirui (2022) has reported the occurrence of multidrug resistance among diverse isolates. Multidrug-resistant variant of *Staphylococcus aureus*, *Salmonella typhi*, and non-typhoidal *Salmonella* (NTS) have been reported to have high minimum inhibitory concentration to cefuroxime (>256), ciprofloxacin (>32), and gentamicin (>256). Moreover, in Ghana, an expansive bacterial isolates have been

found in teaching and regional hospitals and it has been observed that these bacterial are resistant to tetracycline (81%), chloramphenicol (75%), cotrimoxazole (72%), and ampicillin (75%). Multiple drug resistance has been found for a combination of ampicillin, tetracycline, cotrimoxazole, and chloramphenicol. Alternatively, a small percentage of resistance has been found for ceftriaxone (6.3%), amikacin (9.9%), and ciprofloxacin (11%) (Kirui, 2022).

Antibiotics are failing due to antimicrobial resistance because bacteria and other microorganisms evolve mechanisms such as drug inactivation, changing of binding sites and reduced drug accumulation to survive exposure to these drugs (Reygaert, 2018; Baylay, 2019). Overuse and misuse of antibiotics, such as unnecessary prescriptions or improper dosages, accelerate this process (Connell, 2003). Resistant pathogens can neutralize antibiotics, prevent their entry, or expel them, rendering treatments ineffective (Li, 2009). As a result, infections become harder to treat, causing persistent illnesses, increased medical costs, and high death rate. Without effective antibiotics, routine medical procedures and surgeries are also at greater risk of complications (Duval, 2018). Molecular docking helps analyze how drugs interact with microbial targets such as enzymes or receptors. Microbes develop drug resistance by altering these targets through mutations, producing enzymes to degrade drugs, or using efflux pumps to remove drugs. Thus, molecular docking predicts how these changes affect drug binding, aiding in understanding resistance mechanisms and designing drugs that can overcome them.

Molecular docking is a technique used to estimate the correct pose of a ligand with its target when the ligand and its target are bound together to form a stable complex

(Dadgostar, 2019). Molecular docking helps overcome antimicrobial resistance by enabling the rational design of drugs that target resistant pathogens more effectively. It predicts how small molecules, such as drugs or plant-derived compounds, bind to specific microbial proteins or enzymes critical for survival or resistance mechanisms (Khan, 2003). By identifying compounds that bind strongly to these targets, docking can guide the development of inhibitors that block resistance pathways, disrupt biofilm formation, or enhance the efficacy of existing antibiotics. This approach accelerates drug discovery and helps develop more precise, targeted therapies to combat antimicrobial resistance.

Even though *S. aureus* is known to be a symbiotic bacterial species of the human body, it certainly causes illness, from skin and soft tissue ulceration to severe illness (Murray, 2022). In 2018, *S. aureus* became the main pathogen-drug combination of antimicrobial resistance, and respectively giving rise to 13,800 and 121,000 deaths in African countries and worldwide (Khan, 2003). Despite the fact that penicillin was very good for treating diseases caused by *S. aureus*, currently, over 90%of human *S. aureus* variant are known to be resistant to penicillin (Chandrana, 2015). The *S. aureus* is now resistant to multiple antibiotics, such as beta-lactams, by producing altered penicillin-binding proteins (PBPs) like PBP2a, which reduce drug efficacy (Miyachiw, 2019). It also employs mechanisms such as biofilm formation, efflux pumps, and production of enzymes to evade antimicrobial action. Resistance in *S. aureus* complicates treatment, leading to severe infections and limited therapeutic options, highlighting the urgent need for alternative strategies and novel antimicrobial agents (Diekema, 2001)

In *S. aureus*, the covalent coupling of proteins to the cell wall peptidoglycan, happens through a preserved mechanism that includes the action of membrane-anchored transpeptidases called sortases (Can, 2012). Sortase A is an enzyme in Gram-positive bacteria, that helps in anchoring surface proteins to the bacterial cell wall (Cox, 2009). These surface proteins contribute to virulence, biofilm formation, and host immune evasion (Druoling, 2014). By facilitating biofilm development and promoting bacterial adhesion to host tissues, sortase A indirectly enhances antimicrobial resistance by shielding bacteria from antibiotics and immune responses (Grund, 2006). Targeting Sortase A with inhibitors is a promising strategy to weaken bacterial virulence and biofilm formation, thereby improving the efficacy of antimicrobial treatments (Hewitt, 2000).

Drug resistant in *E. coli* is found predominantly in the hospital settings, in the community, and surrounding environment (Galindo-Mendez, 2002). In *E. coli*, antimicrobial resistance is a great global health issue, driven by its potential to get and disseminate genes that are very resistant. Key mechanisms include the production of beta-lactamases and carbapenemases, which are known to deactivate beta-lactam antibiotics, as well as efflux pumps, reduced membrane permeability, and target site mutations. *E. coli* can develop resistance to multiple drug classes, including fluoroquinolones, aminoglycosides, and tetracyclines, making infections difficult to treat. Its role as both a commensal organism and a pathogen facilitates the expansion of genes that are resistant between diverse bacterial species, further exacerbating antimicrobial resistance crisis. Beta-lactamases and DNA gyrases

have been considered as strong resistance strategies being present in most resistant *E. coli* (Galindo-Mendez, 2002).

DNA gyrase, an essential bacterial enzyme, plays an important function in antimicrobial resistance by portraying as the target of fluoroquinolone antibiotics (Sikora & Zahra, 2023). This enzyme, along with topoisomerase IV, helps maintain DNA supercoiling necessary for replication and transcription (Bilung, 2018). The mutations in the genes that encodes DNA gyrase is known to change the enzyme's structure, reducing fluoroquinolones binding affinity and leading to resistance (Robiscsek, 2006). Additionally, overexpression of efflux pumps or reduced membrane permeability can limit drug access to DNA gyrase. Inhibiting DNA gyrase is a critical strategy for combating bacterial infections, but resistance mechanisms highlight the need for novel inhibitors.

Beta-lactamase enzymes play a central role in antimicrobial resistance by breaking down beta-lactam ring in the antibiotics, including penicillins, cephalosporins, and carbapenems, making them not to be very effective (Can, 2012). These enzymes hydrolyze the beta-lactam ring, which is essential for the antibiotic's ability to inhibit bacterial cell wall synthesis. Some bacteria produce beta-lactamases or carbapenemases, which makes them resistant to many beta-lactam antibiotics (Lowry, 2016). The widespread production of beta-lactamases in bacteria like *E. coli* and *K. pneumoniae* has made infections harder to treat, emphasizing the need for beta-lactamase inhibitors and alternative therapies.

The large increase in bacteria that have become resistant to antibiotics and the difficulty that comes when treating infections caused by these bacteria has spurred

researchers to look for novel antibacterial compounds and also come out with new methods of fighting bacterial infections (WHO, 2020). Compounds sourced from plants are known to have an activity as antibiotic resistance altering chemicals (Bilung, 2018). Combining plant extracts with antibiotics is important because it can enhance the effectiveness of antibiotics, particularly against resistant pathogens. Plant extracts, rich in bioactive compounds, may work synergistically with antibiotics to increase bacterial susceptibility, inhibit biofilm formation, and target multiple pathways simultaneously (Grund, 2006). This combination can lower the required antibiotic dose, reducing aftereffects and lessening the progress of resistance. It also provides a potential strategy to combat multidrug-resistant bacteria and improve the efficacy of existing antimicrobial therapies.

Turmeric is an herbal plant that is used as a food spice and in conventional medicine. Traditionally, turmeric has been used to treat skin infections, respiratory infections, and joints (Prasad & Aggarwal, 2011). Turmeric exhibits antimicrobial activity primarily due to curcumin, its active compound. Curcumin disrupts microbial cell membranes, inhibits protein synthesis, and interferes with microbial signaling pathways. Turmeric is effective to counter a wide range of bacteria and some viruses (Sharifi-Rad, 2020). Its ability to target antibiotic-resistant strains and inhibit biofilm formation makes it a very good antimicrobial agent for preserving food and potential therapeutic applications (Ramesh, 2022).

Garlic is used in folk medicine to treat diseases such as tuberculosis (Murray, 2022). Garlic exhibits strong antimicrobial activity because it contains sulfur compound like allicin, which is released when garlic is mashed. Allicin is known to destroy

cell membranes of microbes and interferes with enzyme functions, making it very efficient against diverse kinds of pathogens, including bacteria, fungi, viruses, and some parasites (Cavallito &Bailey, 2015). Garlic can inhibit the activity of *Escherichia coli* by concentrating on DNA gyrase (Connell, 2003).The antimicrobial properties of garlic is as a result of its potential to inhibit biofilm formation and combat antibiotic-resistant strains, making it a promising natural alternative or adjunct to conventional antimicrobial treatments (Shaikh, 2015).

Studies have revealed that cinnamon has an antibacterial effect on bacteria (Trinh, 2015; Surassmo, 2016 &Yap, 2015). Cinnamon exhibits antimicrobial activity primarily due to its essential oils, particularly cinnamaldehyde and eugenol (Tung, 2010). The compounds found in cinnamon destroys the cell membrane of microbe, impedes enzyme activity, and hinder the metabolism in microbes which causes the death or inhibition of bacteria, fungi, and viruses (Langeveld, 2014). Cinnamon is known to be very efficient against diverse kinds of pathogens, including *E. coli* (Vangalapato, 2012). Its antimicrobial properties make it a valuable natural preservative in food systems and a potential alternative for managing infections (Nazzaro, 2013).

1.2 Statement of Problem

Currently, bacteria have developed resistance to ampicillin which is part of WHO first-line antibiotics and are administered for mild cases (Ventola, 2015). It is expensive to produce new antibiotics, however, turmeric, cinnamon and garlic are inexpensive spices used in our locality (Biondo, 2023). Thus, this current study combines ampicillin with ethanolic extracts of turmeric, garlic, and cinnamon in

other to enhance the inhibitory effect of ampicillin so that this first-line antibiotic can be reused.

1. 3 Research Questions

1. What is the phytochemical constituent of cinnamon, turmeric and garlic?
2. What is the inhibitory effect of ethanolic extracts of cinnamon, turmeric, and garlic on ampicillin-resistant bacteria?
3. What is the inhibitory effect of combining garlic, cinnamon, and turmeric with ampicillin on ampicillin-resistant *Escherichia coli* and *Staphylococcus aureus*?
4. What is the binding mode and possible interactions of the bioactive compounds in cinnamon with Extended-Spectrum-Beta-Lactamase?
5. What is the binding mode and possible interactions of the bioactive compounds in turmeric with sortase A?

1.4 Main Objective

To study the inhibitory effects of ethanolic extracts of garlic, turmeric, and cinnamon on ampicillin-resistant *Escherichia coli* and *Staphylococcus aureus* and also carry out molecular docking analysis of the active compounds in these extracts.

1.4.1 Specific objectives

1. Investigate the phytochemical constituent of the ethanolic extracts of cinnamon, turmeric, and garlic.
2. Determine the antimicrobial activity of different concentrations of ethanolic extracts of garlic, turmeric, cinnamon, and ampicillin.

3. Determine the antimicrobial activity of a combination of different concentrations of ethanolic extracts of garlic, turmeric, and cinnamon and ampicillin.
4. Conduct molecular docking analysis of the bioactive compounds in ethanolic extracts of turmeric and cinnamon with sortase A in *Staphylococcus aureus* and beta-lactamase in *Escherichia coli* respectively.

1.5 Significance of the Study

As a result of the great amount of resistance to western antibiotics by microorganisms, it is very important to use combination therapy in order to achieve efficient bactericidal activity. Different plant extracts can be combined or a standard antibiotic can be combined with the plant extracts. The combination of plant extracts with antibiotics against bacteria which are resistant will have distinct mode of action and it will result to new choices for the treatment of diseases. Combining plant extract with standard antibiotics can be used to enlarge the antimicrobial spectrum in other to stop the rise of mutants that are resistant, to lessen toxicity, and thereby possessing antimicrobial activity bigger than what would have been expected from each antimicrobial drug separately.

CHAPTER TWO

LITERATURE REVIEW

2.1 Antimicrobial Resistance

Antimicrobial resistance happens when a microbe becomes resistant to an antimicrobial drug that was once efficient in treating a disease caused by the microbe (WHO, 2020). Drug resistance has been known to occur in all kinds of microbes (Tanwar, 2014). The antimicrobial resistance process occurs naturally; however, it mostly occurs from inappropriate use of antibiotics and infection treatment (Tanwar, 2014). Antibiotic resistance is known to occur naturally by genetic mutation or by the transfer of the resistance from one microbe to another microbe (Kraker, 2016).Random mutations causes resistance to emerge spontaneously but random mutation can also occur from the horizontal gene transfer of resistant genes (Magiorakos, 2015). Antibiotics that have been used for a long time promote the selection of mutations that can make antibiotics ineffective (Dabour, 2016). The type of antimicrobial resistance that occurs frequently is caused by altered or inherited genes that give bacteria the propensity to colonize the antibiotic-associated killing mechanism (CDC, 2019). Clinical resistance happens when there is failure of many therapeutic strategies, in which microbes that are usually vulnerable to treatment develop resistance after surviving the effects of the treatment. Bacteria can transfer genetic catalyst which are used for resistance in all the categories of acquired resistance so that the antimicrobial resistance can spread to other species of bacteria (MacGowan, 2017). Antimicrobial

resistance pathogens are known to be the principal causes of morbidity, mortality, and economic burden in rich countries as well as poor countries (Cai, 2019).

2.2 Mortality, Morbidity, and Economic Burden Due to Antimicrobial Resistant Bacterial

Morbidity is the state of having a specific illness while mortality is the state of being subject to death (Kraker, 2016). Globally, almost 700,000 people die yearly from diseases caused by antimicrobial resistance pathogens and many of these numbers are understated as a result of substandard reporting and surveillance studies (Kraker, 2016). The Center for Disease Control has confirmed that 23,000 humans experience death yearly because of disease caused by bacteria which are resistant and it infects over 2 million people globally (Takahashi, 2016). According to a study done in India, antimicrobial resistance infections are responsible for 60,000 neonatal deaths annually (Davies, 2013). Hannan (2013), reported that multidrug-resistant bacteria like *Escherichia coli* isolated from human blood samples cause 40% of neonatal deaths. While 37 out of 78 deaths in newborns were caused by antibiotic-resistant bacteria (Saleem, 2009)

Immunocompromised individuals have been reported to have the highest mortality rates in poor nations as compared to developed nations. Multiple patients on one bed, contaminated drinking water, poorly equipped rooms, patient overcrowding, etc. are the major risk factors in public hospitals in poor countries (Llor and Bejernum, 2014). Globally, colossal sums of money are spent to combat diseases caused by bacteria which are resistant to antimicrobials. The European Union alone spends over 1.5 billion euros yearly in combatting diseases caused by

antimicrobial-resistant bacteria (Davies, 2013). Also, a study reported that the US alone spends over 20 billion dollars yearly to cure 20 million individuals who have acquired antimicrobial resistance infections (Humpton, 2013). In underdeveloped nations, one reason causing the increase in antimicrobial resistance internationally is the rise in the prescription of antimicrobials (Humpton, 2013). It is estimated that over 700, 000 million deaths occur yearly as a result of bacteria that are resistant to antimicrobials and the problem represents a menaces to global public health (Drame, 2020). Dadgostar (2019), reported that at least 2.8 million people in the US contract pathogens that are antibiotic-resistant annually while 35,000 people die as a result. It is estimated that 55 billion dollars are lost in productivity and health care expenditures. The WHO has indicated that by the year 2050, a total of 350 million deaths will be caused by antimicrobial resistance (Chanel, 2020). Research has indicated that antimicrobial resistance is caused by selective pressure, wrong use of antibiotics, and inheritance of genes from other bacteria that make them resistance.

2.3 Causes of Antimicrobial Resistance

Microbes are living entities that change over time. The survival of microorganisms largely depends on multiplying, thriving, and spreading out efficiently and quickly (Murray, 2022). Microbes evolve to adapt to their surroundings to ensure that they survive. Selective pressure is one-way microbes develop resistance to antimicrobials and in this case, if the bacteria have a resistant gene and in the presence of antimicrobials, the bacteria will be eliminated or persist. The bacteria that will survive will reproduce and outnumber other types of bacteria that are

present in the bacteria population (Donkor, 2019). During the bacteria replication, mutations occur, and these mutations help a bacteria strain to carry on while interacting with antibiotics (Samuel, 2019). Bacteria sometimes inherit genes from some bacteria species that make them resistant to antibiotics. Even when antibiotics are administered properly, their use creates a selective pressure for resistant microbes. However, sociocultural factors work to make the growth of antibiotic resistance very fast. Antibiotics used inappropriately increases the development of resistant microbes (Duong, 2021).

Doctors frequently diagnose infection using little or wrong information and prescribe an antibiotic that is broad-spectrum instead of using specific antibiotics which will be more effective: these situations help boost selective pressure which then gives rise to antimicrobial resistance (Presinaci, 2016). Thus, antimicrobial resistance can be prevented by proposing an international convention on antimicrobial resistance, having a global tracking system, using antibiotics as prescribed by a doctor (Melon, 2015).

2.4 Prevention of Antimicrobial Resistance

One of the public requests for a coordinated global action to address antimicrobial resistance is a submission of a global convention on drug resistance. Global tracking system is an idea that has been floated but it has not been put into action. On a global scale, more information is required to identify and assess patterns in antimicrobial resistance. This system will identify areas where antimicrobial resistance is very high and give the information required for program evaluation, interventions, and modifications made to fight antimicrobial resistance (Chanel,

2020). Antimicrobial resistance is sped up by misuse, abuse, and inadequate infection control.

Using antibiotics as prescribed by a doctor, following a doctor's prescription when taking antibiotics, keeping vaccines up to date, washing your hands frequently, and staying away from sick individuals are ways to stop antimicrobial resistance from spreading (Melon, 2015).

Proposing an effective national action plan to fight antimicrobial resistance, and tracking diseases with antimicrobial resistance are practical steps policymakers can follow to stop the spread of antimicrobial resistance while health personnel can help stop the spread of antimicrobial resistance by keeping their hands and instruments clean to prevent infections and following the recent recommendations during the prescription of antimicrobials (Weese, 2010).

Lastly, the agricultural industry can help prevent the spread of antimicrobial resistance by administering vaccines to animals to reduce the need for antimicrobials; use antibiotic alternatives, and implementing best practices at all levels of production (Ito, 2017**).** Some bacteria are resistant to multiple antibiotics. Multi-drug resistant is known to affect the health care industry and it has been categorized into primary, secondary, and clinical multi-drug resistance (Loeffler, 2002).

2.5 Multi-Drug Resistance

One of the problems that affects health care globally is multidrug resistance (MDR). Frequent exposure to antimicrobial medications is causing bacteria to be resistant

to antibiotics. In the past decade, diseases caused by microbes have increased significantly and this has resulted in the rise of antimicrobial resistance (Das, 2014). Microbes that are resistant to chemotherapeutic treatments are said to be exhibiting resistance to multiple drugs (Nikaido, 2020). Multiple drug resistance is known to be a natural occurrence among bacteria species, its prevalence is rising for a variety of reasons such as the usage of unspecified drugs, the use of filthy sanitary settings, and undeveloped medical facilities (Barbosa, 2019). Multi-drug resistance is a common phenomenon in *Staphylococcus aureus* and hence this multi-drug resistance has been categorized into primary, secondary, and clinical multidrug resistance (Loeffler, 2002). The survival of any microbe has eventually resulted in widespread antimicrobial resistance and this resistance has many mechanisms such as target change, drug deactivation, lowered drug absorption, and drug efflux (Reygaert, 2018)

2.6 Mechanism of Drug Resistance in Bacteria.

Bacteria that are resistant to drugs undergo four main mechanisms, that is: target change, drug deactivation, lowered drug absorption, and drug efflux (Chinemerem, 2022).

During drug inactivation, penicillin-resistant bacteria form beta-lactamase in order to deactivate penicillin G (Reygaert, 2018).

Methicillin-resistant *Staphylococcus aureus* modifies penicillin-binding protein as an example of binding site change or target modification (Gholiof, 2022).

Ribosome protection proteins are a defense mechanism present in various bacteria. The proteins protect the cells of the bacteria from the actions of antibiotics that want to stop protein production by impeding the ribosomes in the cell (Reygaert, 2018). The ribosome protection proteins attach themselves to the ribosomes of the cell of the bacteria through a process that changes their conformational structure. Because of this, the ribosomes continue to generate proteins that will help the cell to survive without the threat of the antibiotics binding to them to stop protein synthesis (Connell, 2003).

Reduced drug accumulation occurs as a result of pumping out the drug to the cell's surface or decreasing the penetrability of the drugs (Li, 2009). Some bacterial groups have pumps within their membranes that are used to pump the antibiotics out of the cell before the antibiotics can cause harm to the bacteria (Morita, 1998 & Aminov, 2007).

Gram-negative bacteria possess lipopolysaccharides layers that block certain chemicals to reduce drug uptake and due to this some bacteria naturally are resistant to some classes of potent antimicrobial drugs (Blair, 2014). *Staphylococcus aureus* is one bacterium that exhibits these mechanisms of drug resistance and it has several roles in disease development. *Staphylococcus aureus* is responsible for food poisoning, sinusitis, skin, and respiratory illness (Tong, 2015).

2.7 The Role of *Staphylococcus aureus* in Disease

S. aureus is a spherical bacterium that is mostly present in the microbiota of the body. It has catalase and nitrate reduction activities and it can live or survive without oxygen because it is a facultative anaerobe (Park & Seo, 2022). As a

commensal, *Staphylococcus aureus* is known to coexist with humans as part of the microbiota. *Staphylococcus aureus* is known to cause sinusitis, food poisoning, skin, and respiratory illness.

Even though *S. aureus* functions as a commensal to colonize 30% of the human population, it can occasionally cause illness (Tong, 2015). *S. aureus* is known to cause bacteremia and infective endocarditis. Also, when the skin and the mucosal barriers are compromised, *Staphylococcus aureus* can cause infections of the skin and soft tissues. *Staphylococcus aureus* infections spread in ways such as touching a pus from an infected sore, touching items used by person who is infected such as towels, bedding, clothing, and skin-to-skin touch with a person who is infected. For those who have had joint replacement, septic arthritis, *Staphylococcal endocarditis*, and pneumonia are three conditions that are dangerous for them (Kuehmert, 2005). *S. aureus* plays a significant contribution in chronic biofilm infections on medical implants (Kavanaugh, 2016). When latent in the body, *S. aureus* can remain undiagnosed for a long time. Also, after the initial signs of illness appear, the carrier is infectious for two weeks and the disease itself stays for some weeks. The untreated illness can be deadly (CDC, 2019). *Staphylococcus aureus* can develop resistance to antibiotics by mutating its chromosomal DNA/RNA (Harshey, 2021). Thus, *Staphylococcus aureus* has developed resistance against drugs such as methicillin, macrolides, tetracycline, and chloramphenicol (Bann, 2015).

2.8 Antimicrobial Resistance in *Staphylococcus aureus*

S. aureus is known to be responsible skin infections and shows toxic shock syndrome (Lowry, 2016). The occurrence of resistant *S. aureus* started rising in

spite of the antibiotics that have been used against it. It has been found that *S. aureus* strains resistant to methicillin are very common. In other to survive, microbes undergo mutations in their chromosomal DNA/RNA which makes them resistant (Harshey, 2021). Methicillin resistance in *S. aureus* is one example. The development of *S. aureus* helps it to become multiple resistant strains (Iskandar, 2020). It has been observed in a recent study that antibiotic-resistant genes are found in the R plasmids which help to bestow multiple resistance to drugs and also influence the lowered use of multiple antimicrobial therapy (Fahrenkamp, 2016). It has been observed that Methicillin-resistant *Staphylococcus aureus* is also resistant to macrolides, tetracyclines, and chloramphenicol (Bann, 2015). Aside from *Staphylococcus aureus* which has developed antimicrobial resistance making its infections difficult to treat, *Escherichia coli* also has developed resistance to antimicrobials (Todar, 2016).

2.9 Importance of *Escherichia coli*

Escherichia coli was known as a commensal bacterium in the colon until strains of it were found as the source of diarrhea in newborns (Todar, 2016). This bacterium has been investigated well and it is responsible for many illnesses (Tooke, 2019 &Tudu, 2022). *Escherichia coli* has been used as an effective biomarker of fecal pollution because it is prevalent in human and animal excreta (Carlos, 2010). Also, since *Escherichia coli* can live outside of the human body for just a small length of time, its cells are used as an excellent indicator organism for checking environmental samples for contamination (Todar, 2016). *Escherichia coli* has been confirmed by studies to stop opportunistic pathogenic microbes from inhabiting the

gut (Tuon, 2023). Some *Escherichia coli* strains have probiotic effects and an example is the Mutaflor isolate of *Escherichia coli* (Sonnenborn, 2016), which has been proven to decrease gastrointestinal colonization by pathogenic microbes and it is very helpful in curing inflammatory disorders by modulating the signal transduction pathways (Lodinova, 1997). *Escherichia coli* has been thought to be responsible for many systemic and localized illnesses in cattle, birds, pigs, and humans. *Escherichia coli*, aside been used as a laboratory workhorse and a colonizer of the intestines, can be a deadly pathogen (Addy, 2004). Infections caused by *Escherichia coli* are very widespread, despite being frequently underestimated. *Escherichia coli* is the leading cause of neonatal meningitis, pediatric diarrhea, and urinary tract infections globally (Nataro &Kaper, 1998). Also, the CDC (2019), has reported that *Escherichia coli* causes 61 fatalities and 73,000 cases of illness yearly in the US. *Escherichia coli* and other similar bacteria are known to make up around 0.1% of the flora of the gut and fecal-oral transmission is the dominant way these pathogens spread diseases. The *Escherichia coli* strain that produced the Shiga toxin caused the outbreak of bacteria that began in Germany in May to June 2011 and it escalated to other eleven European countries, including some parts of North America. *Escherichia coli* O104:H4 is known to be the strain that led to a prominent outbreak in Europe and it resulted in 4,321 infections, 885 hemolytic uraemic syndrome, and 50 fatalities. It is known that children under the age of seven in undeveloped countries have a 50% chance of dying from diarrhea infection (Gillen, 1991). In Ghana, gastroenteritis and UTI cases caused by pathogenic *Escherichia coli* are documented (Addy, 2004; Djie-

Maletz, 2008). Thus, all these diseases and deaths caused by *Escherichia coli* are because *Escherichia coli* has developed resistance against antimicrobials used to treat its infections (Voukeng, 2012).

2.10 Antimicrobial Resistance in *Escherichia coli*

Antimicrobial resistance happens when microbes resist the action of drugs that are used against the microbes. Different strains of *Escherichia coli* that have become resistant to antimicrobials are deemed a great threat to global health (Voukeng, 2012 & Li, 2009). Drug resistance in *E. coli* is found in hospital settings, in the community, and in the environment. *Escherichia coli* has used many protective strategies to lessen the effects of drugs used against it. Extended-spectrum beta-lactamase, fluoroquinolones, and carbapenemases are deemed as great resistance tactics that are present in bacterial strains that have become resistant (Younis, 2018). Mobile genetic elements are considered to possess the greatest contribution to the transfer of resistance genes among bacterial cells. Transposons, another mobile genetic element, is considered one of the great sources of transmission of resistance (Fabio, 2020). Collectively, mobile genetic element helps in exchange, acquisition, and sharing of resistance genes. Antimicrobial resistance in *Escherichia coli* has been confirmed globally and there are differences in its resistance pattern. The most dominant phenotypes of *Escherichia coli-resistant* clones are the CTX-M extended-spectrum beta-lactamase, carbapenems, colistin-resistant, and ST-131 *Escherichia coli*. The resistant variants that have been listed are mostly found in Sub-Saharan Africa, China, and South Asian countries. To combat antimicrobial resistance in bacteria, a combination of plant extract and

standard antibiotic can be employed and turmeric is one herb that can be used because it has antibacterial compounds and has been used in households to treat infections (Todar, 2016).

2.11 Turmeric

Turmeric, a perennial herb, is part of the Zingiberaceae family. It is primarily farmed in India, Pakistan, and China (Dashan, 2017). Turmeric has been used as a household therapy to treat a lot of infections (Vasconelos, 2018 & Martinson, 2020). Turmerone, curdione, and ar-turmerone are responsible for the characteristics and aroma of the turmeric rhizomes. Curcumin is responsible for turmeric's distinctive yellow color (Todar, 2016). The turmeric rhizome contains fat, protein, minerals, carbs, moisture, and essential oils. The essential oils contain compounds such as borneol, curcumin, cineol, sabinene, zingeberene and sesquiterpines (Khan, 2003). There are phytochemicals in turmeric which is responsible for its therapeutic effects.

2.11.1 Phytochemistry of turmeric

Turmeric is known to contain different phytochemicals, such as curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, tumerones and turmeronols (Todar, 2016). Curcumin is known to be the active component of turmeric and it makes up to 2 to 5% of the herbal spice. The yellow color of the spice and its therapeutic effect is as a result of the presence of curcumin in it.

Some volatile compounds identified in turmeric are zingeberene, curlone, turmerone, and ar-turmerone whilst the cucuminoids are part of the non-volatile

substances. Curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingiberene, curcumenol, and curcumol are the main compounds present in turmeric that confers antimicrobial activity to the turmeric plant (Vermassen, 2019).

2.11.2 Antimicrobial activity of turmeric

Chandarana (2015), reported that curcuminoids, one of the phenolic chemicals found in turmeric, are potent against *Staphylococcus aureus*. Turmeric's antibacterial properties are due to its essential oil, alkaloids, curcumins, turmerol, and valeric acid. Odhav (2010), reported that the mechanism behind the antimicrobial property of turmeric is a result of the formation of hydrogen bonds with membrane proteins which then destroy the cell membrane, resulting in the collapse of the cell wall and the electron transport chain. Moreno (2015), reported that the antimicrobial activity of the phenolic complexes in turmeric is caused by the inactivation of cellular enzymes which is dependent on the rate at which substances penetrate the cell and change its penetrability. Najah (2017), revealed that turmeric extract exhibited good antibacterial activity against *Helicobacter pylori* and that the turmeric extract was effective in inhibiting the bacteria with a zone of inhibition of 7.7mm. Also, Niamsa (2009) described that an aqueous extract of turmeric inhibited *E. coli, S. aureus, K. pneumonia*, and *S. epidermidis* at minimal concentrations. According to Mari (2012), turmeric extract inhibited the activity of *E. coli*, and *V. cholera* with a zone of inhibitions of 7mm to 15mm and 10mm to 15mm respectively whilst Kasta (2020), described that ethanol extract of turmeric inhibited *E. coli*, and *S. aureus* microbiota with zones of inhibitions of

15.88mm, 15.63mm, and 15.22mm respectively at a concentration of 500mg/ml. Thus, turmeric is known to impede the growth of *S. aureus* in vitro but in silico molecular docking analysis can be done to predict how the major bioactive compounds in turmeric bind and interact with their target in *S. aureus*.

2.11.3 Molecular docking of the major bioactive compounds in turmeric against sortase A in *Staphylococcus aureus*

To establish infection, *Staphylococcus aureus* develops a lot of virulence factors which then promote the adhesion of the bacteria, invasion of the tissue, and the evasion of the host defense. The adherence of the bacteria and its invasion which are mediated by surface proteins are the first stages necessary for *Staphylococcus aureus* infection (Mann, 2016). Furthermore, surface proteins are important for shunning the immune response from the host. Sortase A, catalyzes the fastening of several virulence-associated surface proteins to the cell wall of *S. aureus* (Mann, 2016). *S. aureus* virulence depends on the fastening of surface proteins by Sortasse A, which suggests that this protein is a suitable mark for antivirulence therapy. Thus, Sortase A inhibitors might stand in for brand-new drugs with cutting-edge tactics in the fight against bacterial illness. Curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingiberene, curcumenol, and curcumol enzyme inhibitors contained in turmeric, have been shown to reduce *Staphylococcus aureus* pathogenicity (Price, 2019).

2.12 Cinnamon

Cinnamon is an herbal spice obtained from the inner back of the shrubs of the genus Cinnamomum and is usually present in the tropical and subtropical parts of

North America, South America, Central America, Southeast Asia, and Australia (Li, 2008). There are about 250 to 359 species of cinnamomum globally. Cinnamon is warm, fragrant, sweet, and very spicy (Suleiman, 2013). The inner back of cinnamon contains the cinnamic aldehyde flavor which is black-brown. Cinnamon has been used to protect food from spoiling since the 16th century (Wemburg, 2018). Cinnamon is used in aromatherapy, perfumes, and conventional and modern medicine. Onderoglu (1999), reported that cinnamon is used to treat stomach disorders and used in cases of nerve weakness, diarrhea, dyspepsia, hyperacidity, vomiting, bloating, and reflux. Cinnamon helps to calm down pain and ensures that the body cells get ample oxygen and this is because cinnamon has substances that make the blood very thin. Also, cinnamon is used to stimulate appetite. Cinnamon is known to be used to treat respiratory infections (Blumenthal, 1998). Khan (2003), reported that cinnamon bark has an impact on insulin (Tisserand, 2014). Cinnamon oil helps to stop the growth of toxic fungi (Anjorin, 2013; Abd El-Aziz, 2015). Jayaprakasha (2017), reported that cinnamon fruit can be used to fight free radicals damage in the body and possibly prevent mutagenesis because they contain antioxidants and phenolics. Cinnamon contains some phytochemicals that are responsible for its antimicrobial activities.

2.12.1 Phytochemistry of cinnamon

Secondary metabolites present in cinnamon make it have antibacterial capabilities (Nazzaro, 2013). Secondary metabolites are known to act as molecules that protect against pathogens and rivals (Efferth, 2011). These secondary metabolites include cinnamaldehyde, cinnamic acid, cinnamate etc. (Vangalapato, 2012) and also lot of

essential oils like trans-cinnamaldehyde, eugenol, L-borneol, cinnamyl acetate, camphor, caryophyllene oxide, b-caryophyllene, E-nerolidol, α-cubebene, α-terpineol, L-bornyl acetate, terpinolene, and α-thujene (Tung, 2010). Each compound's existence and quantity vary based on the part of the herbal plant (Rao, 2014). These phytochemicals present in cinnamon are responsible for its antimicrobial activity on *Escherichia coli.*

2.12.2 Antimicrobial activity of cinnamon

Cinnamon is known to contain several phenolic chemicals that have antibacterial properties like any other plant (Langeveld, 2014). The three most important phenolics in cinnamon are cinnamaldehyde, cinnamate, and cinnamic acid (Vangalapato, 2012). Trans-cinnamaldehyde which is present in the bark of the herb is responsible for its antibacterial activity (Rao, 2014). Utchariyakiat (2016), reported that cinnamon exerts antimicrobial activity on bacterial, yeast, and fungal species. Brodhurst (2018), reported that cinnamon exerts an antibacterial effect on some microbes and that cinnamon has favorable potential when it is combined with antibiotics against drug-resistant bacteria. In an in vitro investigation by Marin and Safi (2014) where 28 plant extracts, oils, and certain antibiotics with a concentration of 5% against bacteria were used, cinnamon demonstrated good efficacy. The presence of phenolics is responsible for most of the antibacterial activity of cinnamon. Lambert (2001), reported that what is responsible for the antimicrobial effect of cinnamon is its ability to cause changes in the permeability of bacterial cell membranes.

Ginovyon (2019), reported that cinnamon extract wields antimicrobial activity by destroying cell envelop and facilitating leakage of intracellular compounds while Nazzaro (2013), described that cinnamon extract demonstrated 81-85% inhibition against *S. aureus, B. cereus, and E. coli*. Price (2019), reported that ethanol extracts of cinnamon can inhibit the development of *S. aureus* with a minimum bacterial concentration of 5% and inhibitory zone of 6.84mm and also inhibited *E. coli* with a minimum bacterial concentration of 10% and inhibitory zone of 5.69mm. Emad (2019), suggested that crude ethanol extract from cinnamon is effective against oral microbes (Vyas, 2015). It is reported that when eugenol is present, *L. monocytogenes* and *E. coli* O157:H7 have been shown to grow more slowly (Blaszyk, 2016). *S. typhimurium, E. coli* O157:H7, and *S. aureus* have been shown to grow less readily when exposed to cinnamaldehyde (Bowles, 1995). While in vitro studies report that cinnamon has antimicrobial activity against several bacteria species, molecular docking analysis can be done to predict how the bioactive compounds in cinnamon interact with their target.

2.12.3 Molecular docking of the major bioactive compound in cinnamon against extended spectrum beta-lactamase

Bacterial resistance brings about numerous health problems in both adults and children (Voukeng, 2016). Usually, beta-lactam drugs are used to cure persistent illness and symptoms. Extended-spectrum beta-lactamase emerged because of bacteria that grow resistant to those antibiotics (Fabio, 2020). This is caused by the quick bacterial mutations when the bacterial are exposed to antibiotics. In this case, beta-lactam antibiotic rings are hydrolyzed by the bacteria using beta-lactamase

enzymes. The extended-spectrum beta-lactamase in *E. coli* has compromised the effectiveness of the recent penicillin and cephalosporin generations (Blaszyk, 2016). The extended-spectrum beta-lactamase in *E. coli* makes the *E. coli* not to be sensitive to beta-lactam drugs hence there is a demand for novel therapeutics because of the loss of the efficacy of drugs and the advent of novel drug-resistant bacteria. The primary compounds in cinnamon are cinnamaldehyde, eugenol, cinnamyl acetate, linalool, and caryophyllene, function as an adjuvant to support the action of synthetic antibiotics against multi-drug-resistant *Escherichia coli*. The cinnamaldehyde, eugenol, cinnamyl acetate, linalool, and caryophyllene in cinnamon target the extended-spectrum beta-lactamase and make it inactive hence the *Escherichia coli* cannot develop resistance to beta-lactam antibiotics (Schlecht, 2015). Consequently, a computational approach based on molecular docking will help in the discovery of beta-lactamase enzyme inhibitors. Garlic is another herbal plant that is known to possess antimicrobial activity against bacteria species like *Escherichia coli*.

2.13 Garlic

Garlic is known to be a member of the Amaryllidaceae family and is thought to have originated in Asia (Subroto, 2021). Garlic is widely used in Egypt, Mexico, China, Europe, and Africa. All the parts of the garlic plant like leaves, bulbs, cloves, and blossoms are used to make concoctions to cure many diseases. Garlic is used as a typical spice and food. A study on the phytochemistry of garlic has reported that allicin and other sulfur-containing compounds are its primary constituent. Diallyl disulfide (DDS), diallyl trisulfide (DTS), and S-allyl cysteine (SAC) are the

main alkaloid that gives allicin its positive effect. Garlic is used in India to treat scabies, dermatitis, graying hair, and lung irritation. Zeng (2017), reported that garlic is used to treat fever and cough. Garlic extract is used in Pakistan to cure fever, and respiratory issues (Dam, 2016). The garlic herb is used to treat fever, liver problems, rheumatism, coli, intestinal worms, and diabetes in Nepal, the Middle East, and East Asia (Zeng, 2017). The ability of garlic to help treat all these infections is because of the phytochemistry of garlic.

2.13.1 Phytochemistry of garlic

Al-Snafi (2013), reported that garlic bulbs have many phytochemicals which include compounds that contain sulfur such as ajoenes, thiosulfinates, vinyldithiins, sulfides, diallyl trisulfide, and others that account for about 82% of the sulfur content of garlic. Alliin, which is the main cysteine sulfoxide, is acted upon by the alliinase enzyme to transform it into allicin after garlic is cut into pieces and the parenchyma is destroyed (Zeng, 2017). S-propyl-cysteine-sulfoxide, allicin, and S-methyl-cysteine-sulfoxide are known to be the main odoriferous molecules of garlic homogenate that have been freshly milled (Zeng, 2017). S-propyl-cysteine-sulfoxide is known to make more than fifty metabolites depending on the temperature and water content. Also, alliinase enzyme can act on a mixture of S-propyl-cysteine-sulfoxide, S-methyl-cysteine-sulfoxide, and alliin to make other known molecules such as allyl methane thiosulfinates, methyl methanethiosulfinte and other corresponding thiosulfinates (Zeng, 2017). The secondary metabolite from cysteine which accumulates in garlic is the S-alk(en)yl-l-cysteine (Souza,

2011). Garlic has several ethnobotanicals uses because of the presence of the phytochemicals.

2.13.2 Ethnobotanical uses of garlic

Garlic is used as a culinary ingredient and condiment because of its pungent smell which is a result of compounds like allicin and diallyl disulfide (Melon, 2015). Garlic is used traditionally to cure hypertension, hair loss, malaria, coughs, wounds, snake bites, pneumonia, asthma, pain, influenza, etc. The numerous benefits that garlic has is because of its anti-diabetic, anti-atherosclerotic, antimicrobial, and antihypertensive potential (Harshey, 2021). Garlic bulb extract is a comprehensive antimicrobial substance with antiviral, antifungal, and antibacterial effects and works well against the majority of enteric species in the Enterobacteriaceae family as well as strains that have developed an antibiotic resistance (Tsao and Yin, 2001; Ross et al., 2001).

2.13.3 Antimicrobial activity of garlic

Garlic has been used to cure human infections and is one of the oldest nutritious and therapeutic herbs known to man. Garlic juices were first identified to exhibit antibacterial action by Louis Pasteur (Younis, 2018). Subroto (2021), reported that methanol and hexane extract from garlic inhibited *E. coli*, and *S. aureus* using agar well diffusion assay. Shaima (2013), reported that aqueous garlic extract was effective as an antibacterial agent against *Staphylococcus aureus* which was resistant to different antimicrobials while Hasib (2022), also described that aqueous extract of garlic inhibited *K. pneumonia*, *E. coli*, and *S. aureus* with minimum inhibitory concentration of 8.33mg/ml, 16mg/ml and 33.3mg/ml respectively.

Allicin in garlic is known to completely stop RNA synthesis while it partially prevents DNA and protein synthesis, which means that RNA is the allicin's main target (Feldberg, 2018). Plant extracts like garlic and others such as turmeric and cinnamon have antibacterial activities on their own and they tend to augment the activity of standard antibiotics when put together (Balestra, 2016).

2.14 Combination of Plant Extracts and Standard Antibiotics in the Treatment of Microbial Infections

Plant extracts that are put together with standard antibiotics tend to either modify the resistance mechanism so that the bacteria can become sensitive to the antibiotics or the antibiotics can act on the bacteria at lower concentrations (Younis, 2018). This method can both lower the dose of the antimicrobial and the aftereffects of the antimicrobial.Vennila (2017), reported that when ethanol extract of *Pinica granatum* rind is combined with ciprofloxacin it exhibits good synergistic activity which results in up to 34-fold reduction of the minimum inhibitory concentration and it re-sensitizes *Klebsiella pneumonia* resistant strain. Also, the combination of the ethanol extract of *Azadirachta indica* and aminoglycosides and carbapenems have been tested for their antibacterial and modulatory effect against *S. aureus*, and *E. coli* (Cristo, 2016). When grape pomace extract is combined with standard antibiotics, it inhibits *S. aureus* and *E. coli* synergistically (Ito, 2017). It has been reported that grape pomace can augment the effect of antibiotics. Carnosic acid in *Rosmarinus officinalis* extracts could act synergistically with gentamicin to inhibit *S. aureus*. Fujita (2005), reported that when beta-lactam antibiotics are combined with baicalein, they exhibit a synergistic effect against drug-resistant *S. aureus*.

Fujita (2005), also reported that *Epigallocatechin gallate* is synergistically active when it is combined with beta-lactams, oxytetracycline, and tetracycline. Nesuta (2016), reported that when the *Geranylated flavones* from *Paulownia tomentosa* are combined with antibiotics they exert synergistic potential to inhibit microbes. It is also reported that the Sicilian plant *Berberis aetnensis* when combined with ciprofloxacin, interacts synergistically to inhibit microbes (Musumeci, 2003). Chloroform extracts of *Berberis aetnensis* are known to lower significantly the minimum inhibitory concentration of ciprofloxacin against *S. aureus*, and *E. coli.* Nascimento (2000), reported that when extracts of clove, jambolan, pomegranate, and thyme are combined with antibiotics, they act synergistically to inhibit resistant strains of *Pseudomonas aeruginosa*. Also, when clove is combined with ampicillin and tetracycline, it enhances the antimicrobial activity of the antibiotics against *Klebsiella pneumoniae* and *Proteus species* respectively (Nascimento, 2000). Balestra (2016), reported that when beta-lactam antibiotics are combined with a-mangostin, the antimicrobial activity of the beta-lactam antibiotics against resistant bacterial strains is improved substantially. Phitakkim (2016), reported that when compounds derived from mangosteen are combined with beta-lactam drugs it inhibits the beta-lactamase enzyme and as a result reactivates the antibiotic.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

The research aims to investigate the inhibitory effect and molecular docking analysis of the combination therapy of the ethanolic extracts of turmeric, garlic, and cinnamon and ampicillin on ampicillin-resistant bacteria.

3.2 Materials

Staphylococcus aureus and *Escherichia coli* which are resistant to ampicillin were the bacteria strains employed in this study. These bacteria species were isolated as pure cultures from the University of Cape Coast Hospital, situated in Cape Coast in the Central Region of Ghana. The bacteria were inoculated on nutrient agar and an antibiotic disc was placed on it and it gave an inhibition zone of 6 mm which means that the bacteria were resistant to the ampicillin since the zone of inhibition was within the resistant zone (≤11) stipulated by the Clinical and Laboratory Standards Institute.

Ampicillin was obtained from the Biomed Pharmacia Pharmacy situated on the University of Cape Coast campus, Cape Coast in the Central Region of Ghana.

Turmeric, garlic, and cinnamon were bought from Abura market in Cape Coast in the Central region of Ghana.

3.3 Experimental Design

The entire research was done by first obtaining the garlic, cinnamon, and turmeric samples. The ethanolic extracts of the turmeric, cinnamon, garlic and ampicillin

solution were prepared and stored until use. The phytochemical analysis of the ethanolic extracts of garlic, cinnamon, and turmeric was performed. The inhibitory effect of the ethanolic extract of garlic, cinnamon, and turmeric was determined and the inhibitory effect for the combination of the ethanolic extract of garlic, cinnamon, and turmeric and the standard antibiotic drugs on the ampicillin-resistant bacteria was carried out. The inhibitory effect of the standard antibiotics on the ampicillin-resistant bacteria was carried out and finally, the molecular docking analysis of the various proteins with their ligands was carried out.

3.4 Ethanolic Extraction of Tumeric, Garlic, and Cinnamon Extract

A total of 500mg of each, turmeric (rhizome), cinnamon (bark), and garlic (bulb) were cut into thin shreds and placed in a thin aluminum foil pan and sun-dried for 8 hours daily for one month until the thin shreds were thoroughly dried. Each of the dried sample was blended in a blender into a fine powder. To prepare the extracts, 80g each of turmeric, cinnamon, and garlic fine powder was added to 500ml of ethanol and kept in an orbital shaker for 24 hours. After 24 hours of shaking, they were sieved through Whitman No.1 filter paper and the filtrates were then heated in a water bath at 40-50 ^{o}C to evaporate the ethanol until a thick paste was formed and weighed 18.685g for turmeric, 28.765g for cinnamon and 8.5g for garlic extract. The extract was stored at 4 ^{o}C until further use.

Each crude extract obtained was separated into two halves. One-half of the crude extract was used to prepare the combination of turmeric, cinnamon, and garlic extract with the standard antimicrobial drug containing 50% of the extract and 50%

of the antimicrobial drug at various concentrations of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml.

3.5 Preparation of Ampicillin Solution

To prepare a stock concentration of ampicillin solution, 1000mg of ampicillin was solvated in 10 ml of distilled water to get a concentration of 100mg/ml. various concentrations of 20mg/ml, 40mg/ml, 60mg/ml, and 80mg/ml were prepared from the stock concentration.

3.6 Method of Sterilization

The Petri dishes and other glassware were cleansed with detergents and rinsed in some changes of distilled water. The petri dishes were autoclaved at 121^{o}C for 15 minutes and then heated in a hot air oven at 70 ^{o}C for one hour to dry. All pipette tips and culture medium that were used were autoclaved for 15 minutes at 121^{o}C to ensure that they were sterilized. The laminar flow cabinet had its surface thoroughly cleansed with methylated spirit.

3.7 Preparation of Nutrient Agar and Broth

Nutrient Agar

The nutrient agar medium was prepared by solvating 28g of nutrient agar powder (Oxioid CM 0003) in 1L of distilled water. The mixture was heated and autoclaved at 121^{o}C for 15 minutes.

Nutrient Broth

An amount of 0.585g oxioid CM 0003 nutrient broth powder was dissolved in 45 ml of demineralized water. The solution was administered in 10ml aliquots into

sterile screw-cap tubes that were autoclaved at 121^{o}C for 15 minutes to ensure their sterilization. After autoclaving, the media was cooled and stored for later use.

3.8 Test for Phytochemicals

3.8.1 Flavonoids

Concentrated sulfuric acid (0.2 mL) and 0.5g of magnesium were added to 1 ml each of the turmeric, cinnamon, and garlic extract and left standing for three minutes. The presence of a pink or crimson coloring that vanishes shows the presence of flavonoids.

3.8.2 Tannins

A few drops of diluted ferric chloride solution were added to 1mL each of turmeric, garlic, and cinnamon extract, and 2ml of water was also added. The presence of tannins was indicated by a green-to-blue coloration (cathechic tannins) or a blue-black coloration (Gallic tannins).

3.8.3 Saponins

The turmeric, cinnamon, and garlic extract (1mL) were added to 1ml of distilled water, and the solution was briskly shaken. The presence of saponins was indicated by twenty minutes of consistent, sustained foam.

3.8.4 Alkaloids

Three to five drops of Wagner's reagent was added to 1mL each of the turmeric, garlic, and cinnamon and the presence of alkaloids is indicated by the formation of reddish-brown coloration.

3.8.5 Anthocyanins

For the anthocyanins, 1 mL of Ammonia and 1mL of HCl were added to 1 ml each of the turmeric, garlic, and cinnamon extract. The presence of anthocyanins is indicated by a color shift from pink-red to blue-violet.

3.8.6 Coumarins

To 1ml each of the turmeric, garlic, and cinnamon extract, 2mL of NaOH (10%) was added. The presence of coumarins is indicated by the presence of yellow color.

3.8.7 Terpenoids

An amount of 2mL of acetic anhydride and sulfuric acid were added to 2ml each of the turmeric, garlic, and cinnamon extract. The presence of terpenoids is indicated by the presence of blue and green rings.

3.8.8 Phenols

An amount of two drops of 5% ferric chloride were added to 1mL each of the turmeric, garlic, and cinnamon extract. The presence of phenols is indicated by deep blue or blue-black hue colors

3.8.9 Quinones

An amount of two drops of conc HCL were added to 1 ml each of the turmeric, garlic, and cinnamon extract. The presence of yellow color indicates quinones.

3.8.10 Oxalate

An amount of two to three drops of glacial acetic acid were added to 2ml of each of the turmeric, garlic, and cinnamon extract. The presence of a greenish-black color indicates the presence of oxalates.

3.9 Antibacterial Sensitivity Testing of the Ethanolic Extracts of Turmeric, Cinnamon, and Garlic only Using Disc Diffusion Method.

The Modified Kirby-Bauer disk diffusion method was used to assess the inhibitory impact of the turmeric, garlic, and cinnamon extract on the ampicillin-resistant bacteria (Cheesbrough, 2006).

Various concentrations of each of the turmeric, garlic, and cinnamon extracts of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, and 100mg/ml were prepared and impregnated on the sterile filter paper disc. The impregnated paper discs were put on the agar petri dish inoculated with the test organism and left for 24 hours, after which the zones of inhibition around the discs were measured using a ruler for the various concentrations of the garlic, turmeric, and cinnamon extract.

3.10Antibacterial Sensitivity Testing Using Ampicillin

The Modified Kirby-Bauer disk diffusion method (Cheesbrough, 2006) was used to study the inhibitory effect of ampicillin on ampicillin-resistant bacteria. Various concentrations of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, and 100mg/ml were prepared from the stock solution of the ampicillin and impregnated into the paper discs. The impregnated paper discs were put on the agar petri dish inoculated with the test organism and left for 24 hours, after which the inhibition zones around the discs were measured using a ruler for the various concentrations of the extract.

3.11 Antibacterial Sensitivity Testing of the Combined Ethanolic extract of Garlic, Turmeric, and Cinnamon and Ampicillin

The Modified Kirby-Bauer disk diffusion method (Cheesbrough, 2006) was used to determine the inhibitory effect of the combined ethanolic extracts of garlic,

cinnamon, and turmeric and the standard antibiotic drug on the ampicillin-resistant bacteria.

Various concentrations of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, and 100mg/ml of the garlic, turmeric, and cinnamon extracts combined with ampicillin were prepared in 50:50 ratios and impregnated on the sterile filter paper disc. The impregnated paper discs were put on the agar petri dish inoculated with the test organism and left for 24 hours, after which the zones of inhibition around the discs were measured using a ruler for the various concentrations of the extract.

3.12 Molecular Docking Analysis

The molecular docking method comprises of the identification of the receptor and ligand, the identification of the active site residues, and the reconstitution of a complex made up of the three-dimensional structures of the receptors and the ligands.

3.12.1 Preparation of macromolecules and ligands

The receptors, which are the protein targets, were obtained from the Protein Data Bank while the ligands were obtained from PubChem and were activated.

3.12.2 Active site detection

With the help of a pocket finder, the active site of the receptor where the ligand will bind was determined.

3.12.3 Molecular docking implementation process

The Gold docking software from Cambridge Crystallographic Data Center was used to dock the ligands with their protein target. The crystal structure and bind

mode were then displayed using Pymol Molecular Viewer programs. The interaction between the protein residues and ligands was depicted using Discovery Studio software from the Cambridge Crystallographic Data Center.

3.13 Data Analysis

All the results were expressed as mean ± standard error of the mean for triplicate determinations and graphs were drawn using the data. Data were submitted to one-way ANOVA analysis using Microsoft Office Excel (2013 Version).

CHAPTER FOUR

RESULTS

4.1 Phytochemical Content of the Ethanolic Extracts of Turmeric, Garlic, and Cinnamon.

The phytochemicals detected in the ethanolic extracts of garlic, cinnamon, and turmeric are represented in table 1. Phytochemical screening of the turmeric reported the presence of flavonoids, saponins, alkaloids, anthocyanin, coumarins, terpenoids, and quinones. Phytochemical screening of cinnamon reported the presence of flavonoids, saponins, alkaloids, anthocyanins, coumarins, phenols, amino acids, and quinones while, phytochemical screening of garlic reported the presence of flavonoids, saponins, alkaloids, coumarins, phenols and quinones.

Table 1: Phytochemical analysis of the ethanolic extracts of turmeric, garlic, and cinnamon extracts.

Phytochemical	Garlic	Cinnamon	Tumeric
Flavonoids	+	+	+
Tannins	-	-	-
Saponins	+	+	+
Alkaloids	+	+	+
Anthocyanins	-	+	+
Coumarins	+	+	+
Terpenoids	-	-	+
Phenols	+	+	-
Amino acids and Proteins	-	+	-
Quinones	+	+	+
Oxalate	-	-	-

+ means present - means absent

4.2 The Inhibitory Effects of Ampicillin, Turmeric Extracts and the Combination of Turmeric Extract and Ampicillin

The comparisons of the various zones of inhibitions of the turmeric extract, ampicillin and the combination of the ampicillin and turmeric extract is presented in figure 1. For the turmeric extract only, the mean zones of inhibitions were 15.25 ± 1.5 mm, 16.5 ± 1 mm, 18 ± 0 mm, 18.5 ± 1 mm, 17.5 ± 1 mm for the concentrations of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml respectively and for the ampicillin only the zones of inhibition were 13.25 ± 0.5 mm, 14 ± 0 mm, 14.25 ± 0.5 mm, 14.5 ± 0 mm, 14.5 ± 0 mm for the concentrations

of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml respectively whilst for the combination of turmeric and ampicillin the zones of inhibitions were 16.5 ± 1 mm, 22 ± 0 mm, 27.5 ± 1mm, 30.5 ± 1 mm, 33.5 ± 1 mm for the concentrations of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml respectively. The *Staphylococcus aureus* was sensitive to the turmeric extract and moderately sensitive to the ampicillin but sensitive to the combination of turmeric and ampicillin.

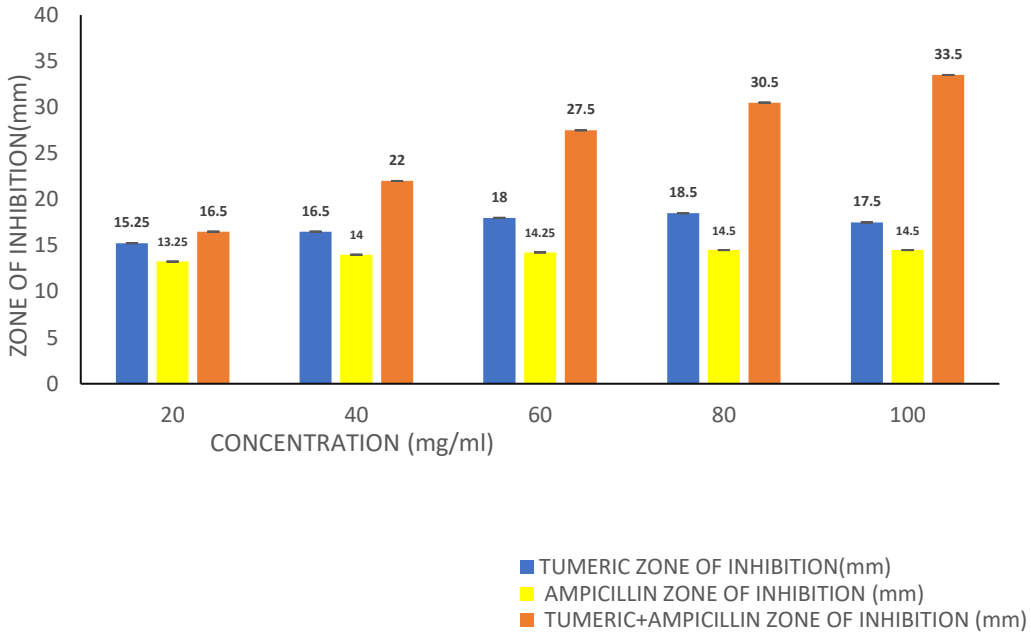

Figure 1: Mean zones of inhibitions of the ampicillin, turmeric extract and the combination of turmeric extract and ampicillin at various concentrations

4.3 The Molecular Docking of the Ligands in Turmeric that Targets Sortase A in *Staphylococcus aureus*

The molecular docking studies predicted the binding mode of curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingiberene, curcumenol, and curcumol with Sortase A protein which is evidenced by a fitness score of 65.72%, 63.46%, 62.37%, 61.80%, 49.19%, 41.26%, and 37.47% respectively.

Table 2: List of ligands in turmeric and their fitness score with sortase A

LIGAND IN TUMERIC	FITNESS SCORE (%)
Curcumin	65.72
Bisdemethoxycurcumin	63.46
Demethoxycurcumin	62.37
Tetrahydrocurcumin	61.80
Zingeberene	49.19
curcumenol	41.26
curcumol	37.47

4.4 The Molecular Docking Studies of Curcumin in Turmeric with Sortase A Protein in *Staphylococcus aureus*

The molecular docking studies of curcumin with the protein, sortase A predicted the binding mode (Figure 2 and 3) which is evidenced by a fitness score of 65.72% and the interactions were stabilized by van der waals interactions, conventional

hydrogen bond, carbon hydrogen bond, pi-pi stacked bond, alkyl and pi-alkyl interactions.

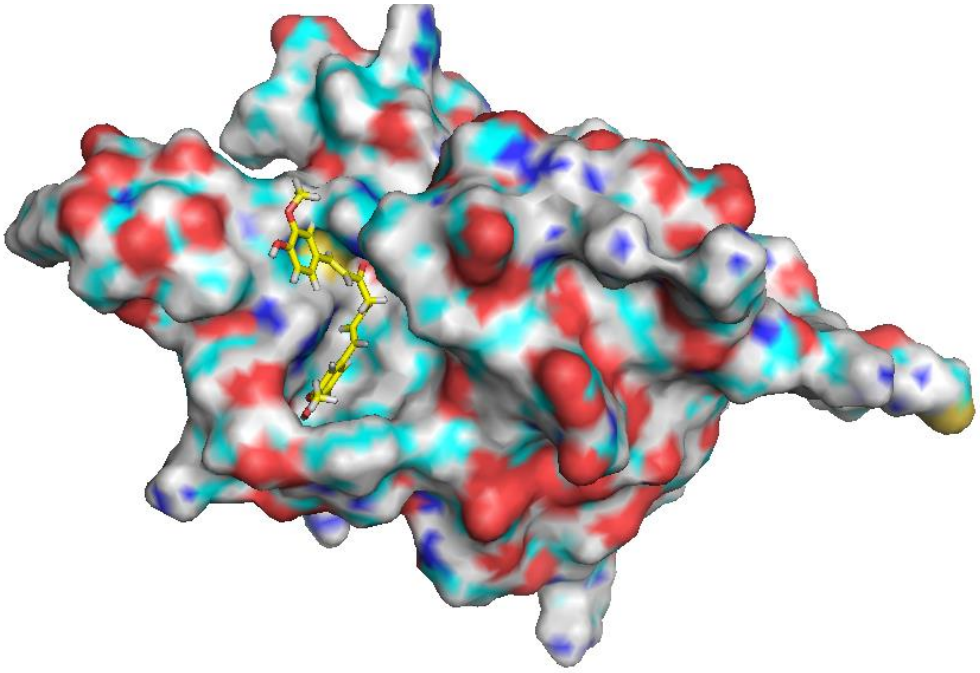

Figure 2: Visualized form of the curcumin-sortase A docked complex

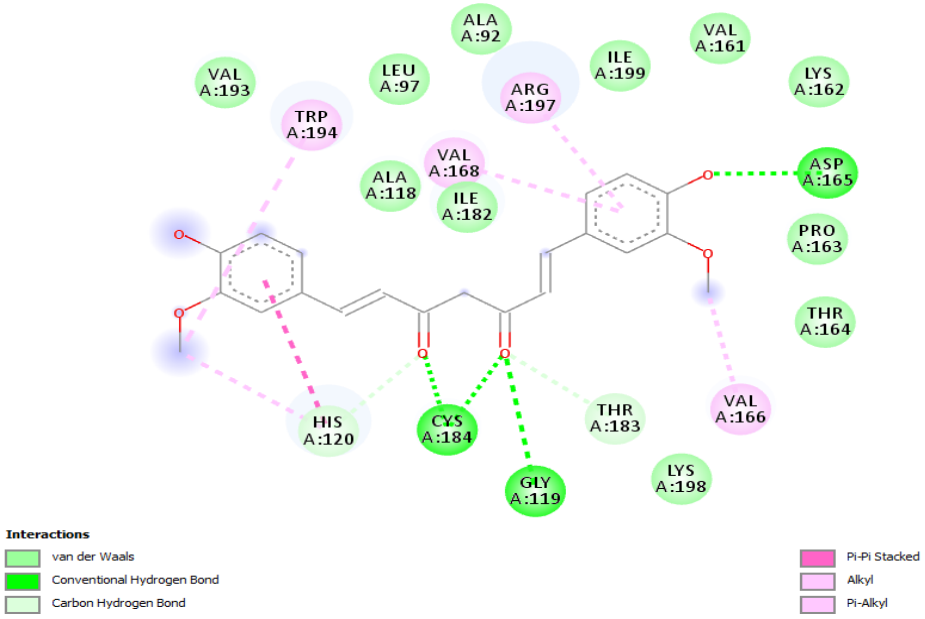

Figure 3: Interactions of curcumin with *Sortase A* residues.

4.5 The Molecular Docking Studies of Bisdemethoxycurcumin in Turmeric with *Sortase A* Protein in *Staphylococcus aureus*

The molecular docking studies of bisdemethoxycurcumin with the protein, sortase A predicted the binding mode (Figure 4 and 5) which is evidenced by a fitness score of 63.46% and the interactions were stabilized by van der waals interactions, conventional hydrogen bond, carbon hydrogen bond, alkyl and pi-alkyl interactions.

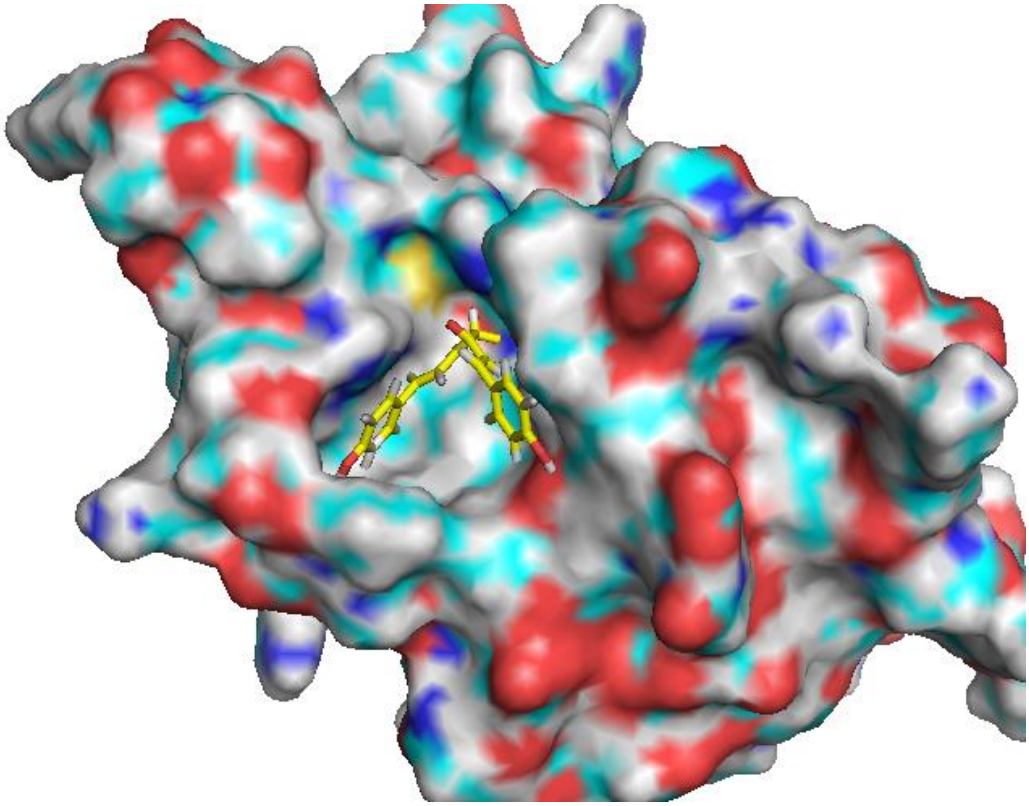

Figure 4: Visualized form of bisdemethoxycurcumin-sortase A docked complex

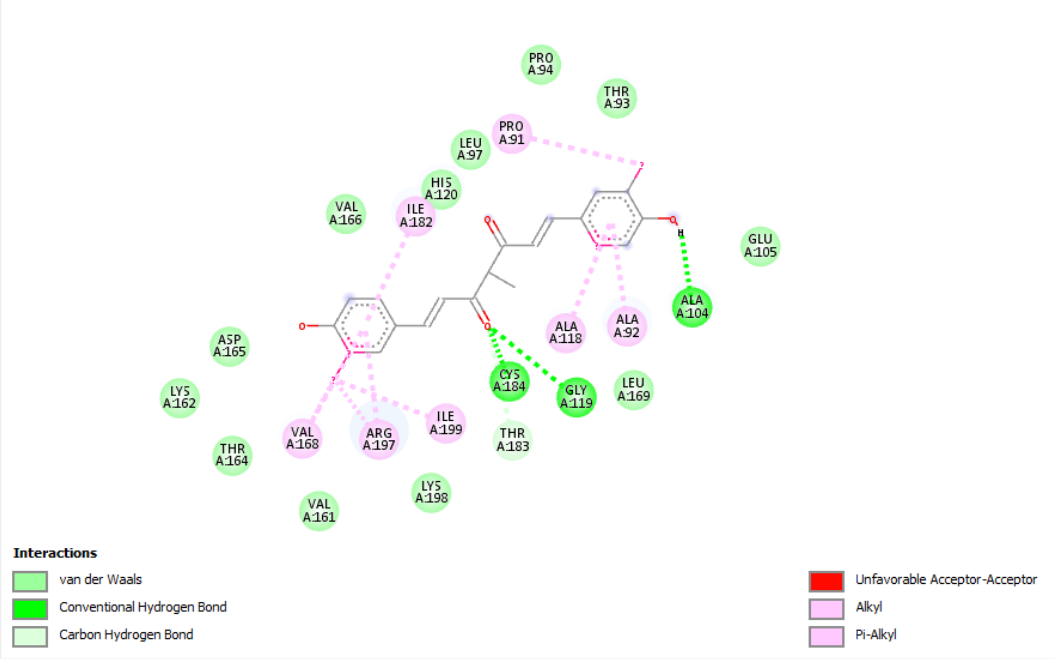

Figure 5: Interactions of bisdemethoxycurcumin with sortase A

4.6 The Molecular Docking Studies of Demethoxycurcumin in Turmeric with Sortase A Protein in *Staphylococcus aureus*

The molecular docking studies of demethoxycurcumin with the protein, sortase A predicted the binding mode (Figure 6 and 7) which is evidenced by a fitness score of 62.37% and the interactions were stabilized by van der waals interactions, conventional hydrogen bond, carbon hydrogen bond, unfavorable bump, alkyl and pi-alkyl interactions.

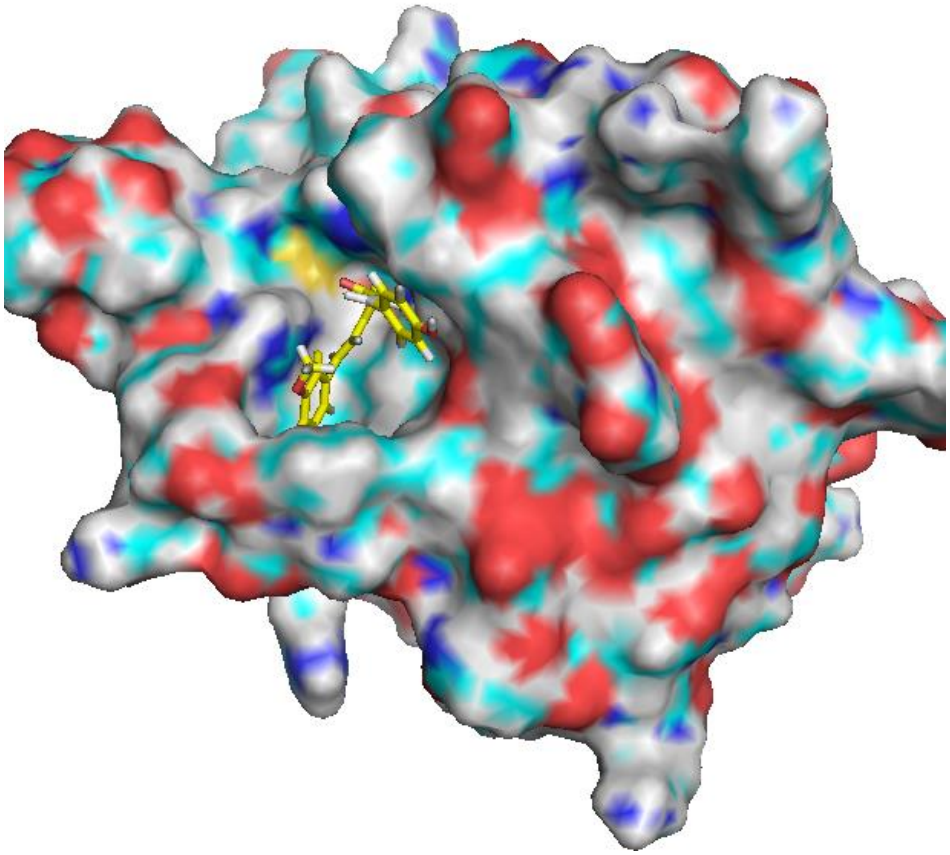

Figure 6: Visualized form of demethoxycurcumin with sortase A

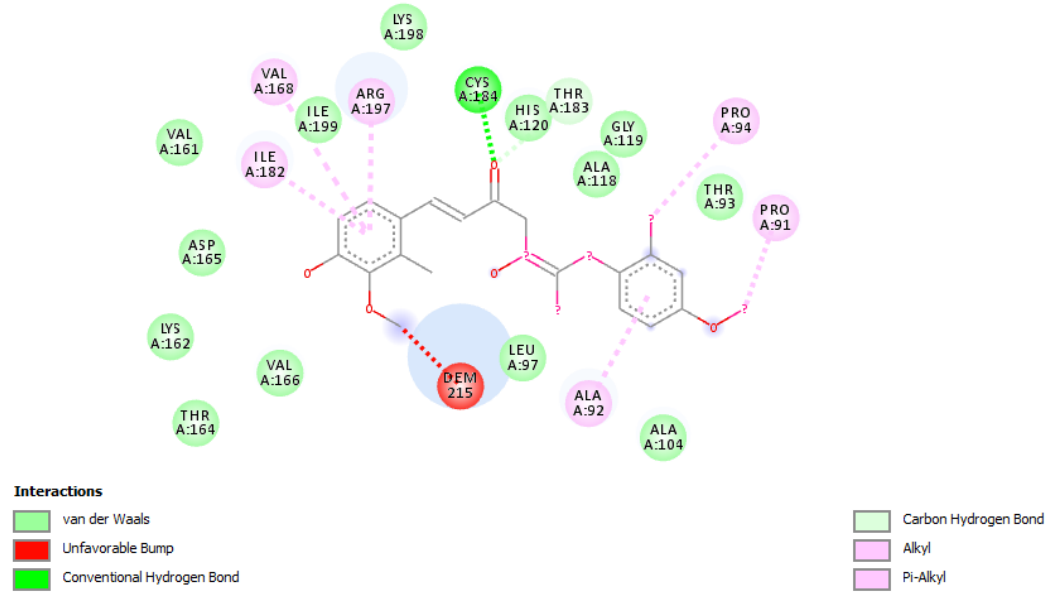

Figure 7: Interactions of demethoxycurcumin with sortase A

4.7 The Molecular Docking Studies of Curcumenol in Turmeric with Sortase A Protein in *Staphylococcus aureus*

The molecular docking studies of curcumenol with the protein, sortase A predicted the binding mode (Figure 8 and 9) which is evidenced by a fitness score of 41.26% and the interactions were stabilized by van der waals interactions and alkyl interactions.

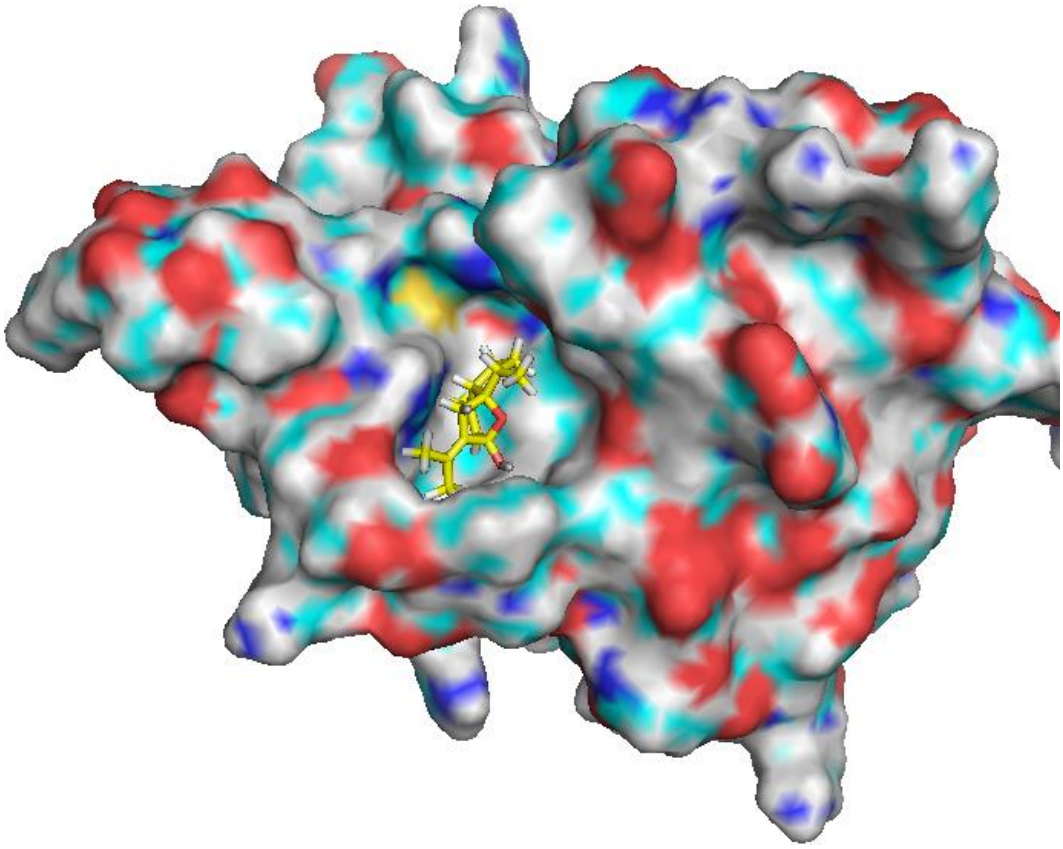

Figure 8: Visualized form of curcumenol-sortase A docked complex

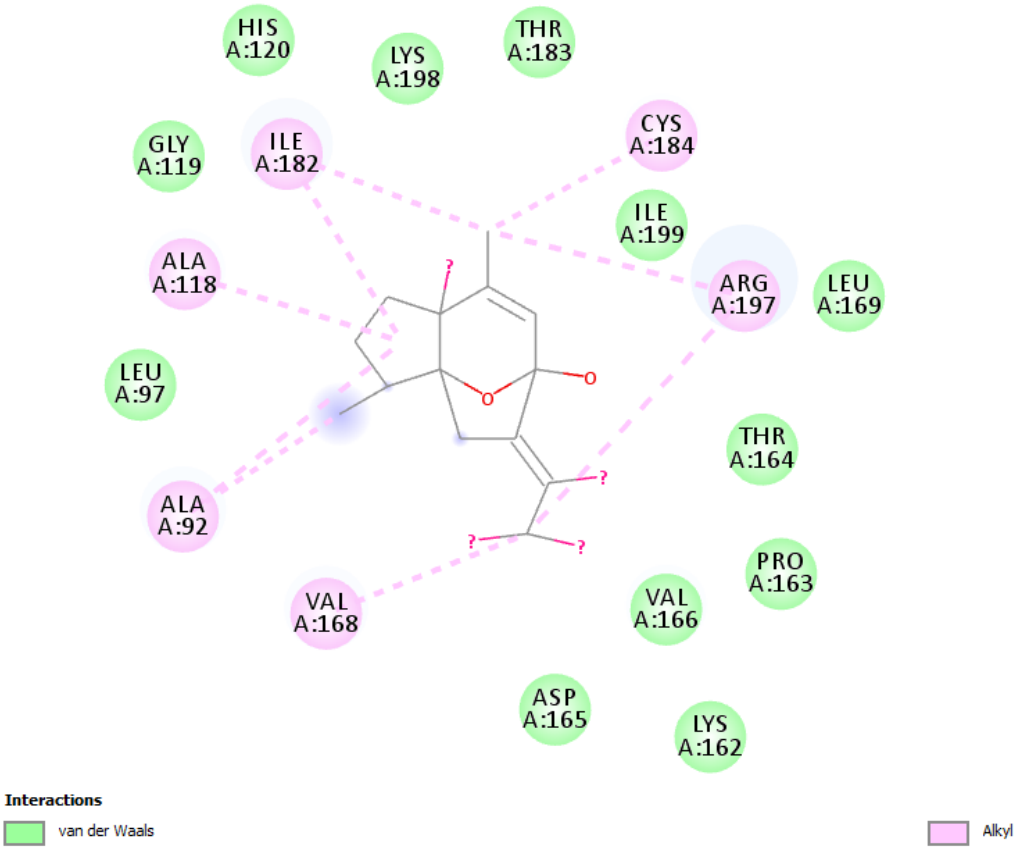

Figure 9: Interactions of curcumenol with Sortase A

4.8 The Molecular Docking Studies of Curcumol in Turmeric with Sortase A Protein in *Staphylococcus aureus*

The molecular docking studies of curcumol with the protein, sortase A predicted the binding mode (Figure 10 and 11) which is evidenced by a fitness score of 37.47% and the interactions were stabilized by van der waals interactions and alkyl interactions.

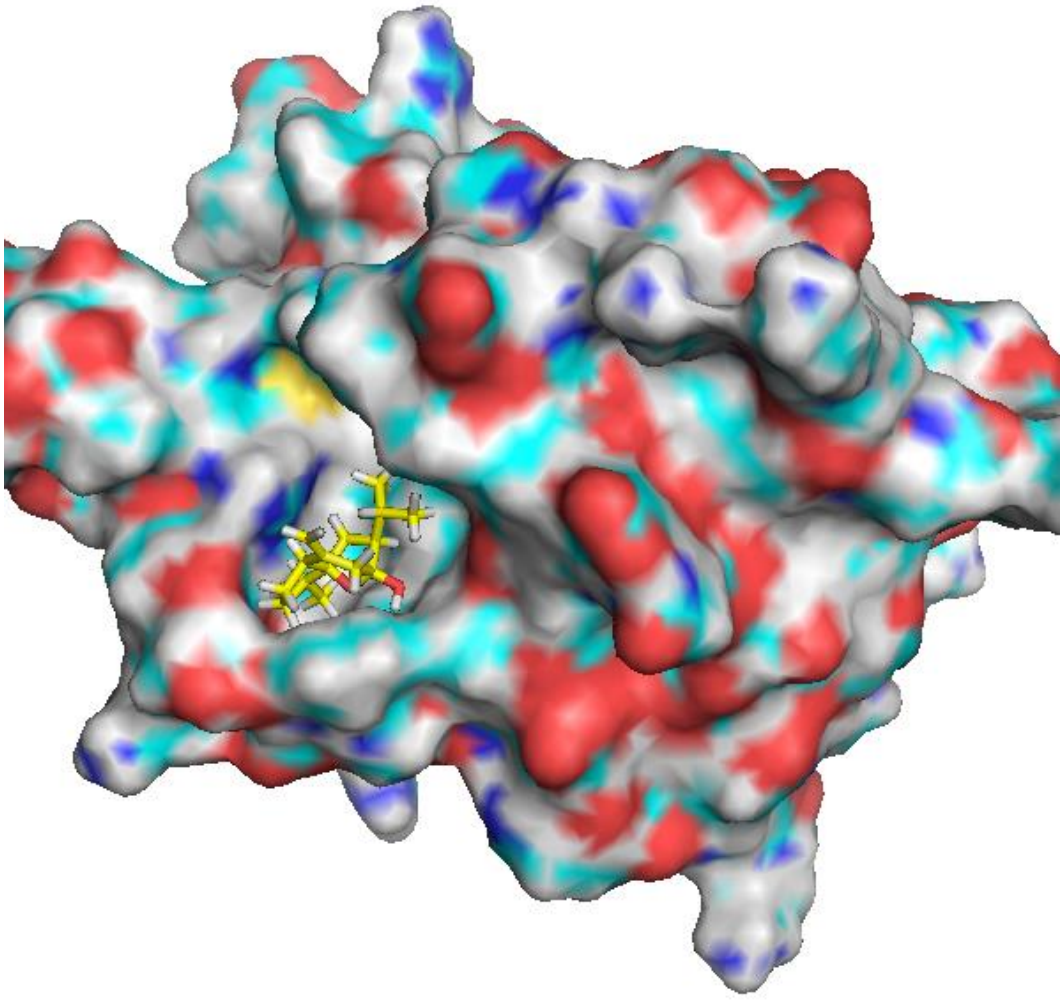

Figure 10: Visualized form of curcumol-sortase A docked complex

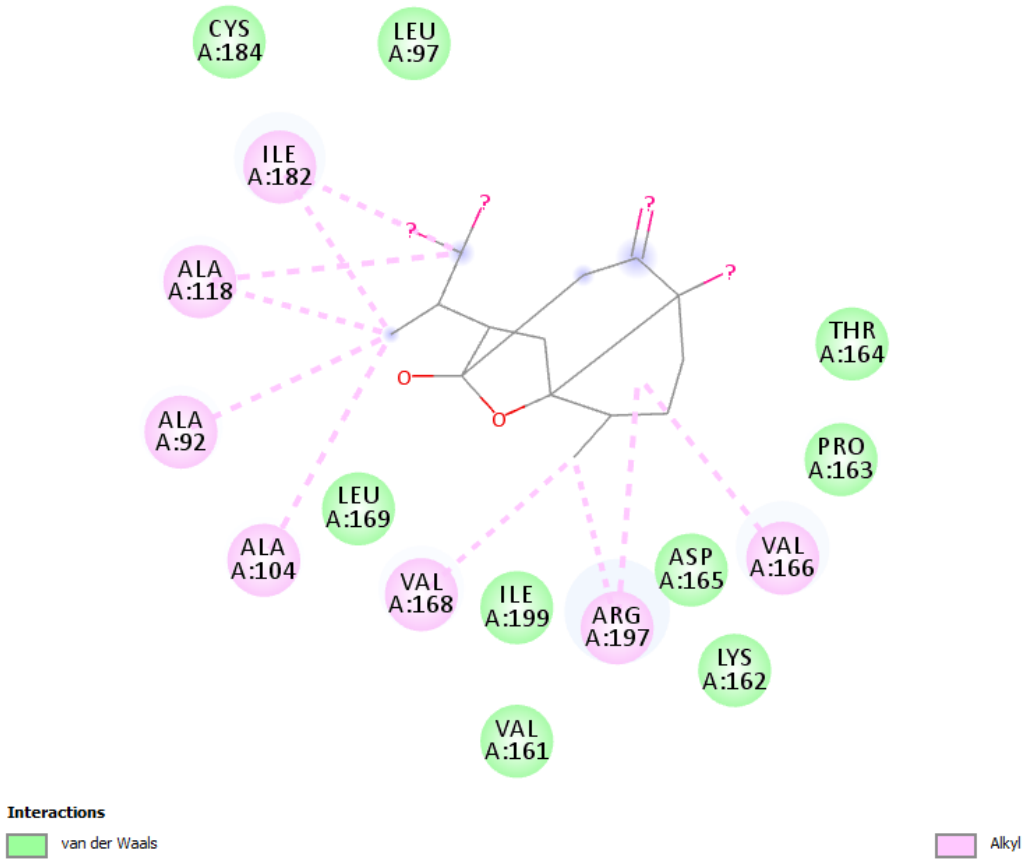

Figure 11: Interactions of curcumol with sortase A

4.9 The Molecular Docking Studies of Tetrahydrocurcumin in Turmeric with Sortase A Protein in *Staphylococcus aureus*

The molecular docking studies of tetrahydrocurcumin with the protein, sortase A predicted the binding mode (Figure 12 and 13) which is evidenced by a fitness score of 61.80% and the interactions were stabilized by van der waals interactions, conventional hydrogen bond, carbon hydrogen bond, pi-cation bond, pi-sigma, alkyl and pi-alkyl interactions.

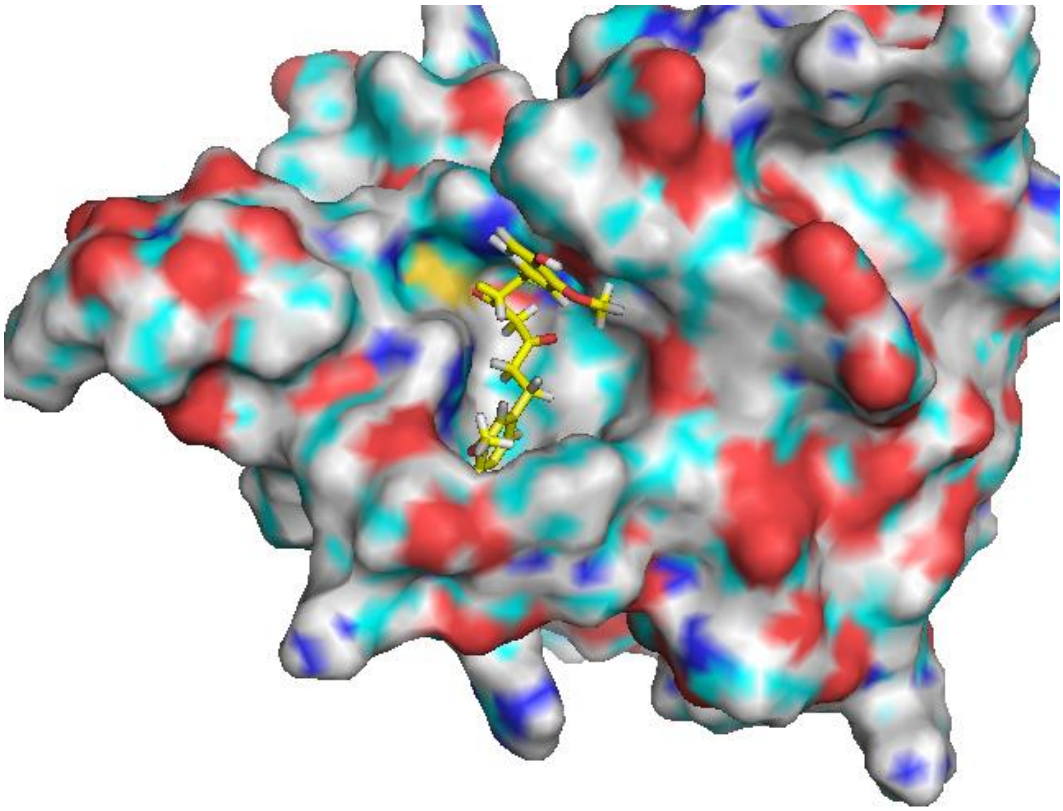

Figure 12: Visualized form of tetrahydrocurcumin-sortase A docked complex

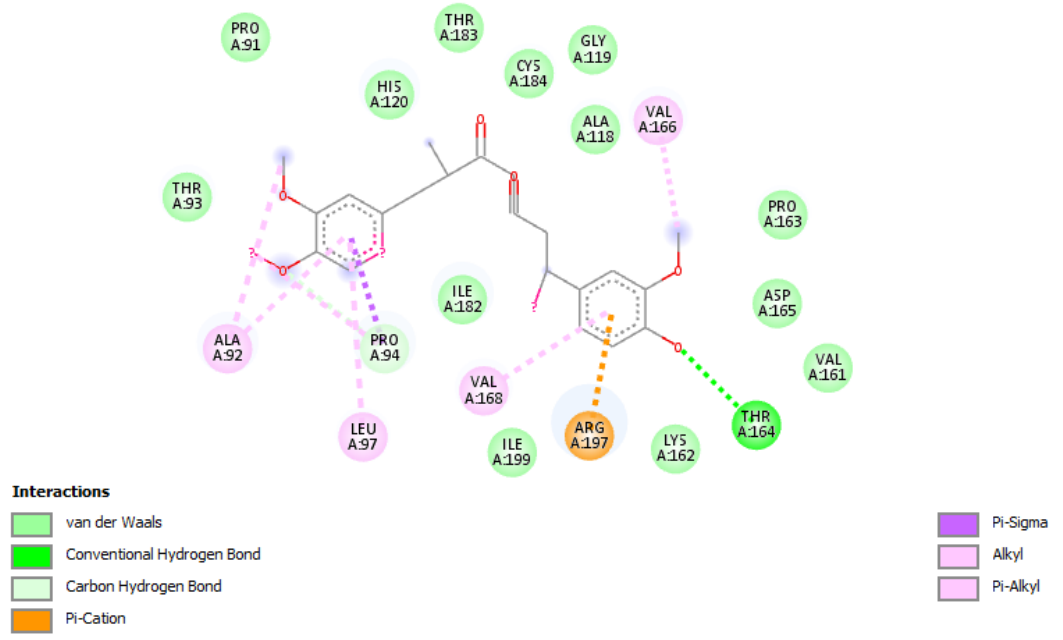

Figure 13: Interactions of tetrahydrocurcumin with sortase A

4.10 The Molecular Docking Studies of Zingeberene in Turmeric with Sortase A Protein in *Staphylococcus aureus*

The molecular docking studies of zingeberene with the protein, sortase A predicted the binding mode (Figure 14 and 15) which is evidenced by a fitness score of 49.19% and the interactions were stabilized by van der waals interactions alkyl and pi-alkyl interactions.

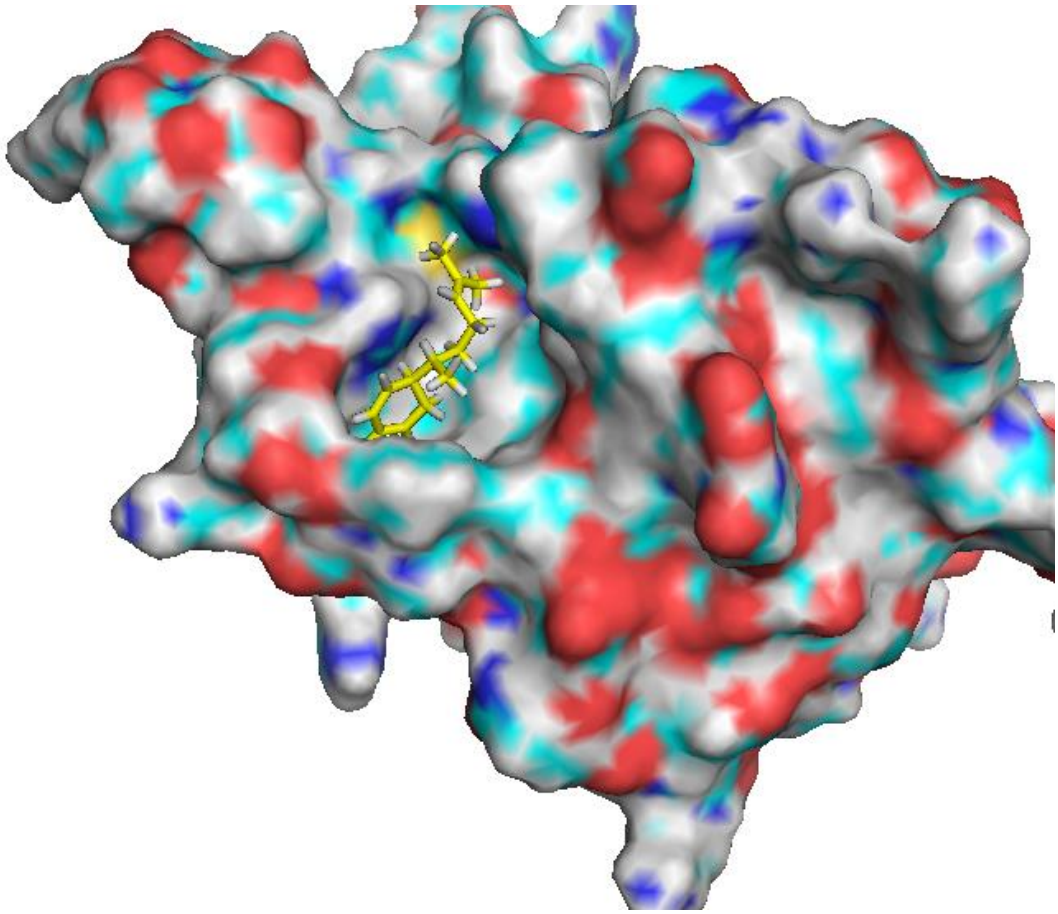

Figure 14: Visualized form of zingeberene-sortase A docked complex

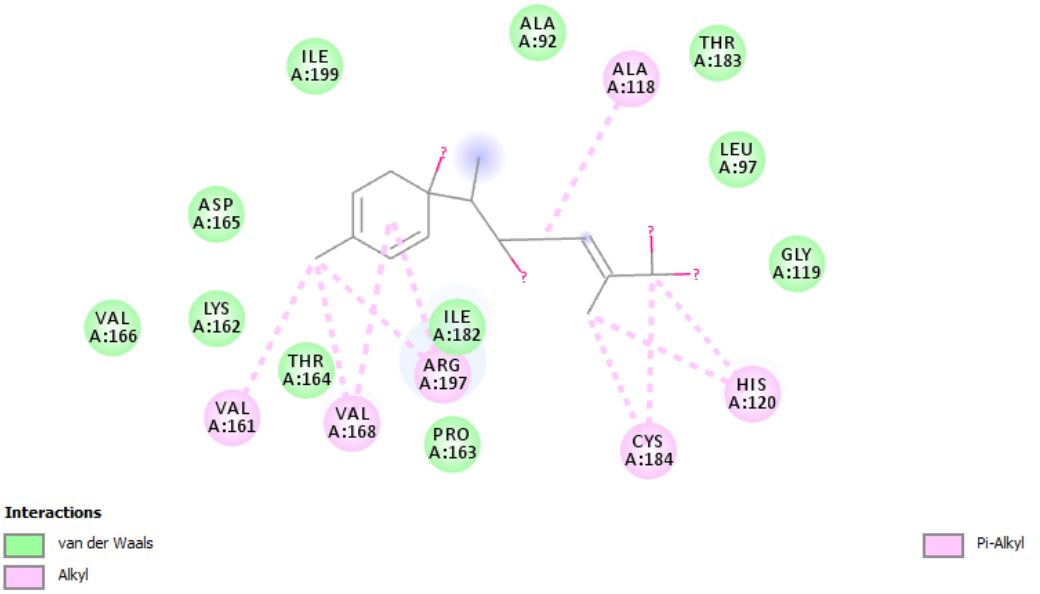

Figure 15: Interactions of zingeberene with sortase A

4.11 Inhibitory Effect of Ampicillin, Cinnamon Extracts and the Combination of Cinnamon Extract and Ampicillin

The zones of inhibitions of the ampicillin, cinnamon extracts and the combination of ampicillin and cinnamon extract at the various concentration is presented in Figure 16. For the cinnamon extract only the mean zones of inhibitions of 11 ± 2 mm, 13 ± 0 mm, 13.5 ± 1 mm, 13.75 ± 1.5 mm, 14.25 ± 0.5 mm were obtained at a concentration of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml respectively and for the ampicillin only zones of inhibition of 13 ± 0 mm, 13.75 ± 0.5 mm, 14.25 ± 0.5 mm, 14.25 ± 0.5 mm, 14.5 ± 0 mm were obtained at a concentration of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml respectively whilst for the combination of cinnamon and ampicillin, zones of inhibitions of 14 ± 4 mm, 17.5 ± 1 mm, 20 ± 0 mm, 23 ± 2 mm, 25.5 ± 1 mm were obtained at a concentration of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and

100mg/ml respectively. The *Escherichia coli* was moderately sensitive to the ampicillin and the cinnamon extract but sensitive to the combination of cinnamon and ampicillin.

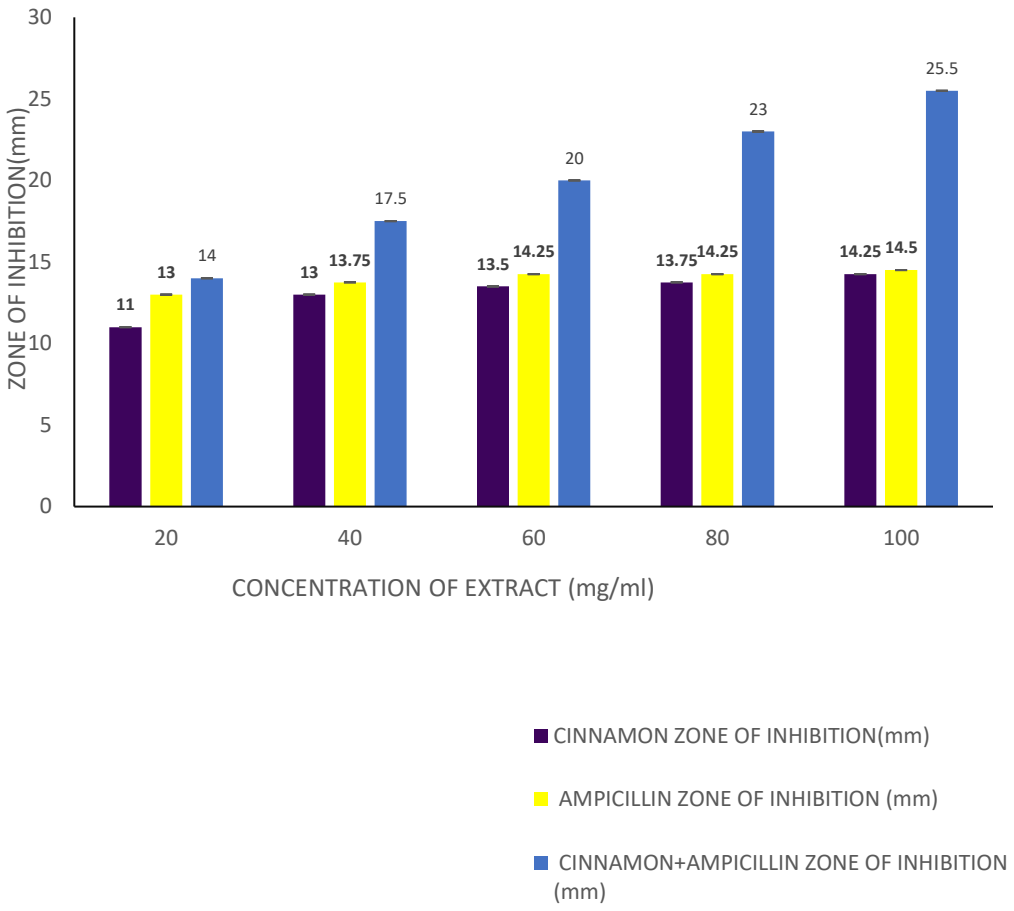

Figure 16: Mean zones of inhibitions of the ampicillin, cinnamon extract and the combination of ampicillin and cinnamon extracts at various concentrations

4.12 The Molecular Docking of the Ligands in Cinnamon that Targets Extended-Spectrum Beta-Lactamase in *Escherichia coli*

Molecular docking analysis of eugenol, cinnamyl acetate, linalool, beta-caryophyllene, and cinnamaldehyde with *extended-spectrum beta-lactamase* predicted the binding mode which are evidenced by a fitness score of 40.64%,39.62%, 38.03%, 36.86%, and 35.25% respectively

Table 3: List of ligands in cinnamon and their fitness score with extended-spectrum beta-lactamase

LIGAND IN CINNAMON	FITNESS SCORE (%)
Eugenol	40.64
Cinnamaldehyde	35.25
Beta-caryophyllene	36.86
Cinnamyl acetate	39.62
Linalool	38.03

4.13 The Molecular Docking Studies of Eugenol in Cinnamon with Extended-Spectrum Beta-Lactamase Protein in *Escherichia coli*.

The molecular docking studies of eugenol with extended-spectrum beta-lactamase predicted the binding mode (Figure 17 and 18) which is evidenced by a fitness score of 40.64% in spite of the smaller molecular weight (164.2g/mol) of the ligand,

eugenol and the interactions were stabilized by van der waals interactions, conventional hydrogen bond, alkyl and pi-alkyl interactions.

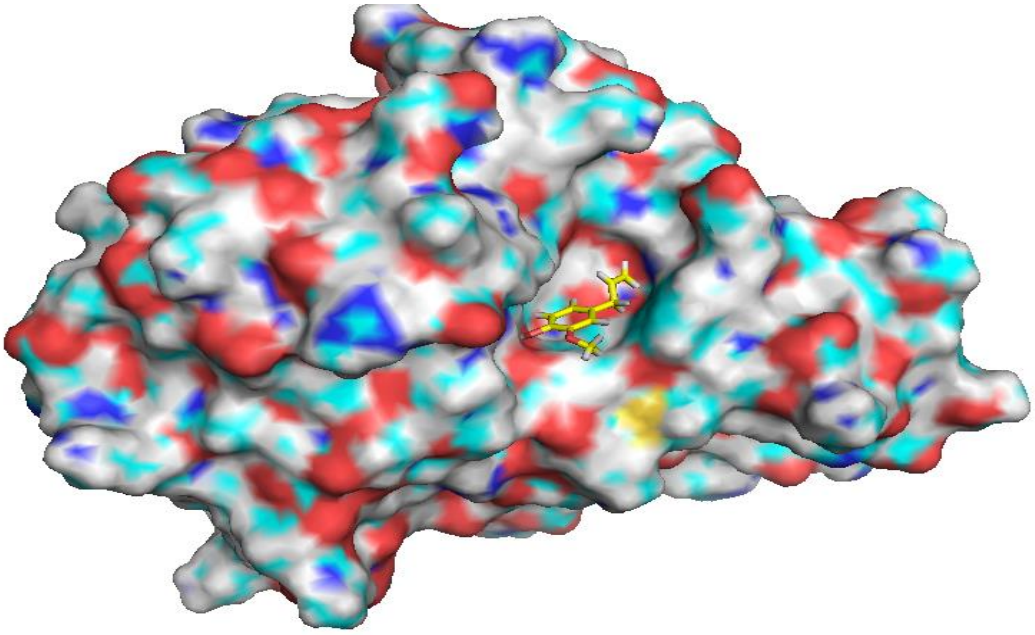

Figure 17: Visualized form of eugenol-extended-spectrum beta-lactamase docked complex

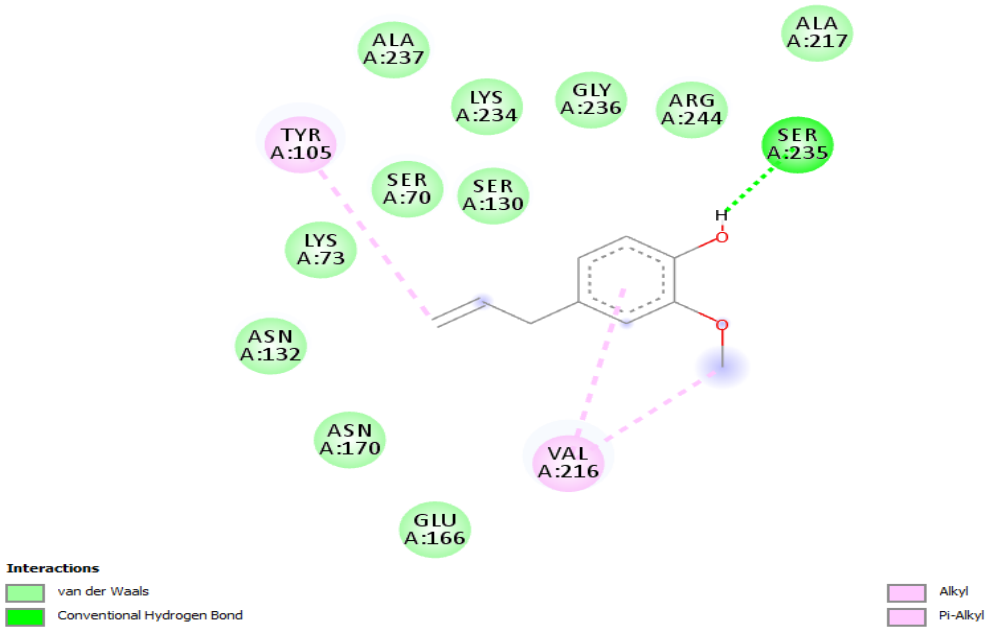

Figure 18: Interactions of eugenol with extended-spectrum beta-lactamase residues

4.14 The Molecular Docking Studies of Cinnamaldehyde in Cinnamon with Extended-Spectrum Beta-Lactamase Protein in *Escherichia coli*

The molecular docking studies of the cinnamaldehyde with the protein, extended-spectrum beta-lactamase predicted the binding mode (Figure 19 and 20) which is evidenced by a fitness score of 35.25% in spite of the smaller molecular weight (132.16 g/mol) of the ligand, cinnamaldehyde and the interactions were stabilized by van der waals interactions, conventional hydrogen bond, pi-cation interactions and pi-lone pair interactions.

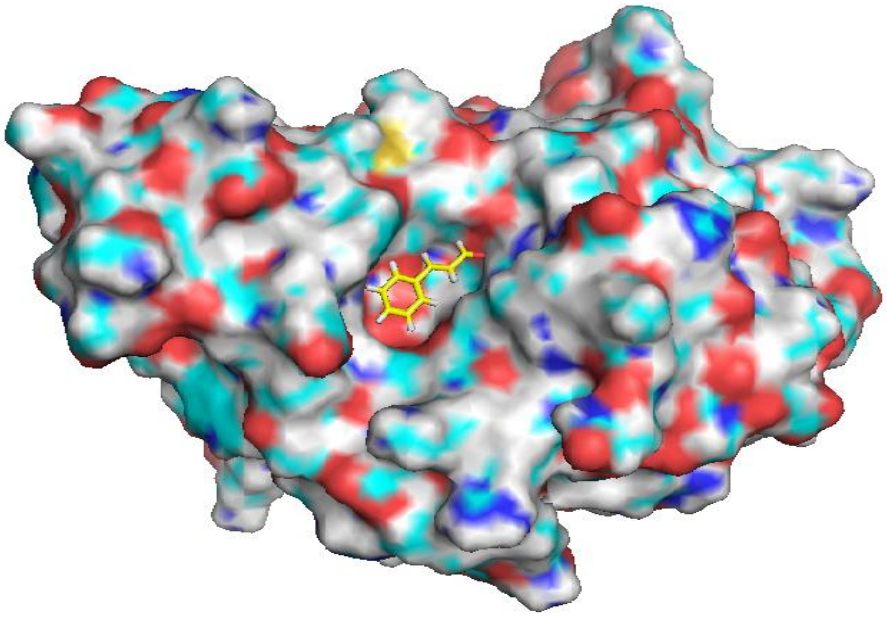

Figure 19: Visualized form of cinnamaldehyde-extended-spectrum beta-lactamase docked complex

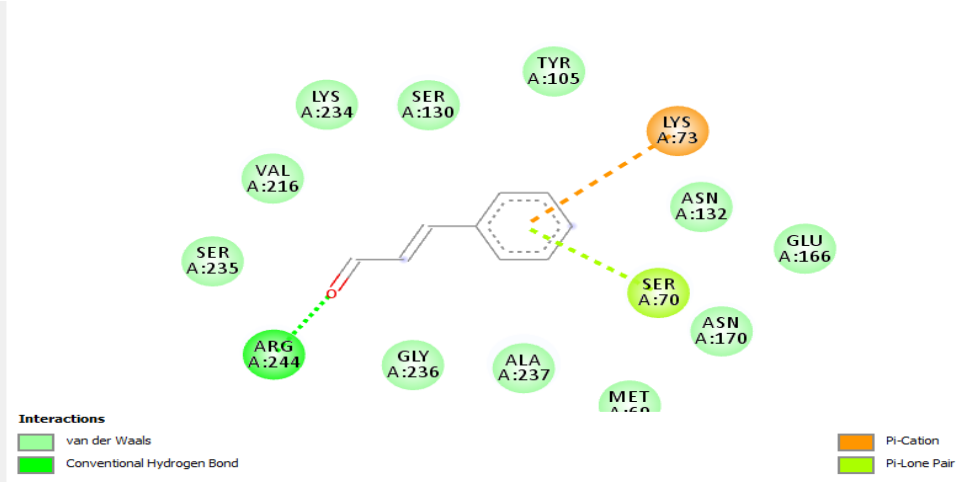

Figure 20: Interactions of cinnamaldehyde with extended-spectrum beta-lactamase residues

4.15 The Molecular Docking Studies of Beta-Caryophyllene in Cinnamon with Extended-Spectrum Beta-Lactamase Protein in *Escherichia coli*.

The molecular docking studies of eugenol with extended-spectrum beta-lactamase predicted the binding mode (Figure 21 and 22) which is evidenced by a fitness score of 36.86% in spite of the smaller molecular weight (204.36g/mol) of the ligand, beta-cryophyllene and the interactions were stabilized by van der Waals interactions, conventional hydrogen bond, unfavorable bump, unfavorable positive-positive, alkyl and pi-alkyl interactions.

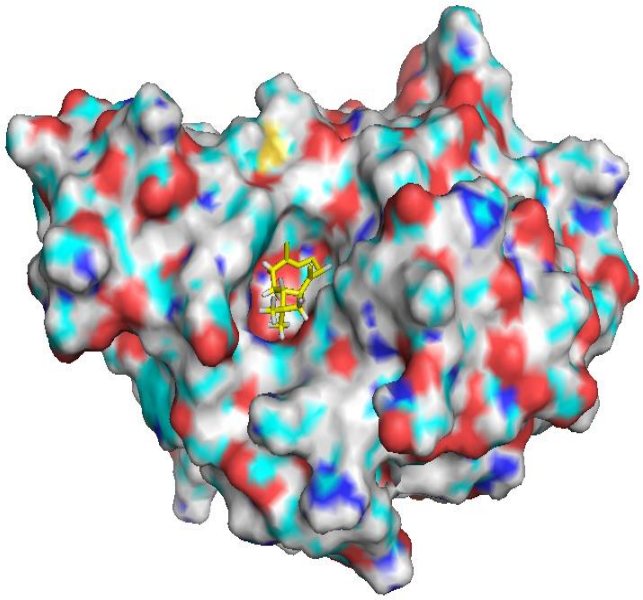

Figure 21: Visualized form of beta-caryophyllene-extendended beta-lactamase docked complex.

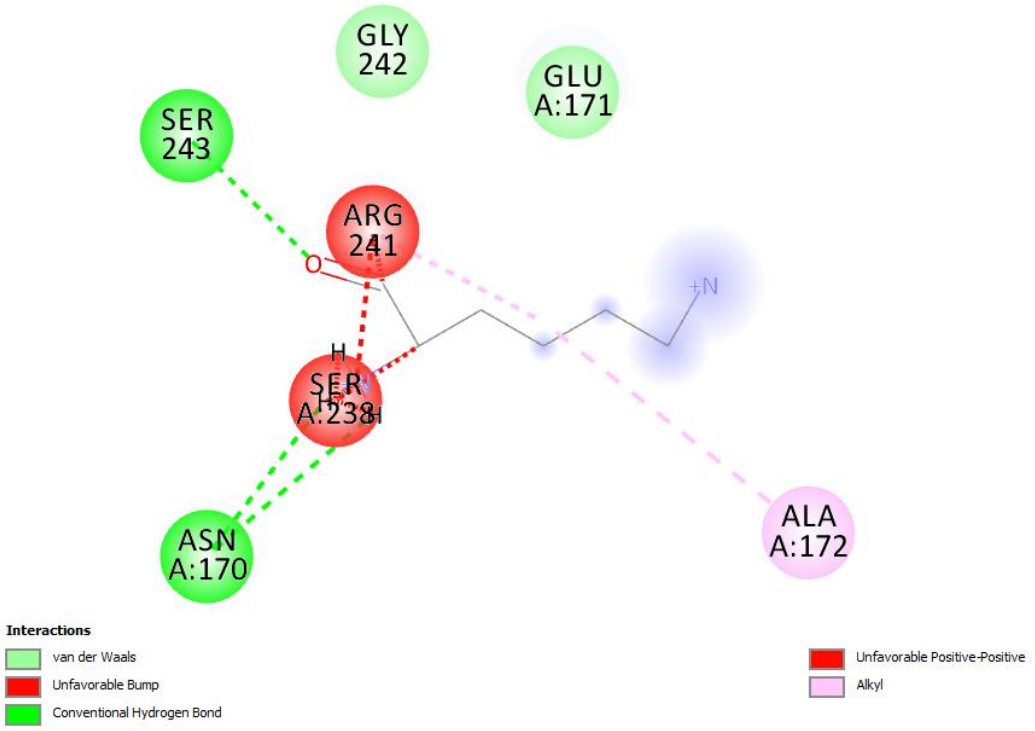

Figure 22: Interactions of beta-caryophyllene with extended-spectrum beta-lactamase

4.16 The Molecular Docking Studies of Cinnamyl Acetate in Cinnamon with Extended-Spectrum Beta-Lactamase Protein in *Escherichia coli.*

The molecular docking studies of cinnamyl acetate with extended-spectrum beta-lactamase predicted the binding mode (Figure 23 and 24) which is evidenced by a fitness score of 39.62% in spite of the smaller molecular weight (176.215g/mol) of the ligand, cinnamyl acetate and the interactions were stabilized by van der waals interactions, conventional hydrogen bond, unfavorable bump, unfavorable positive-positive and alkyl interactions.

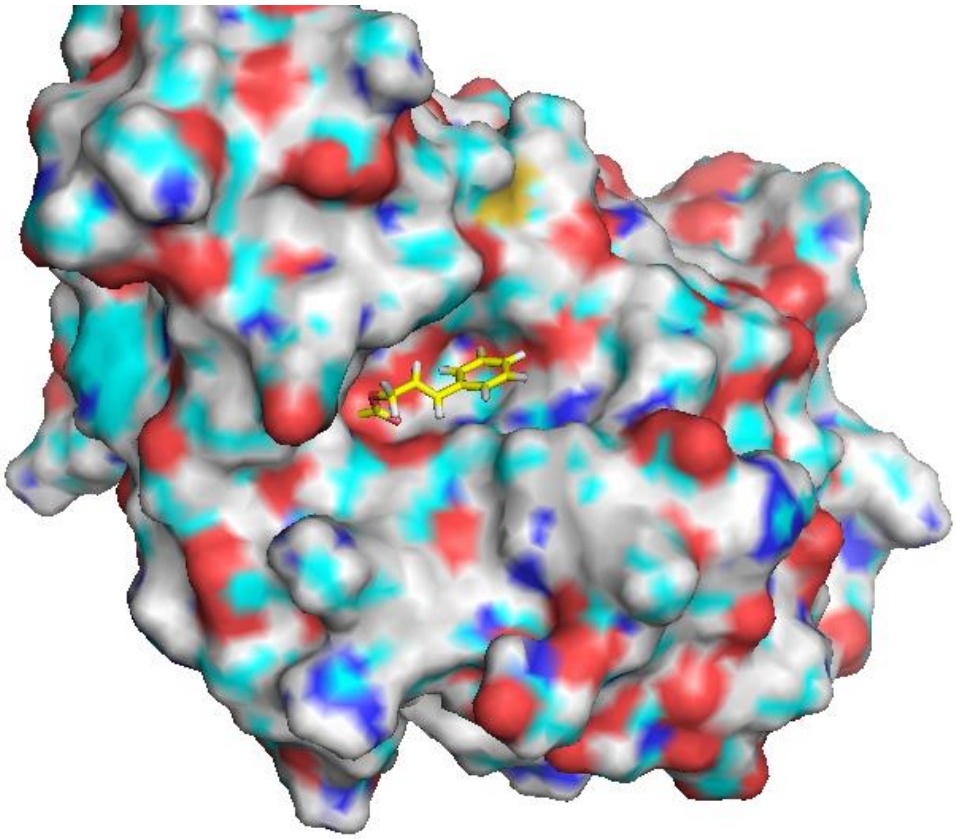

Figure23: Visualized form of cinnamyl acetate-extended-spectrum beta-lactamase docked complex

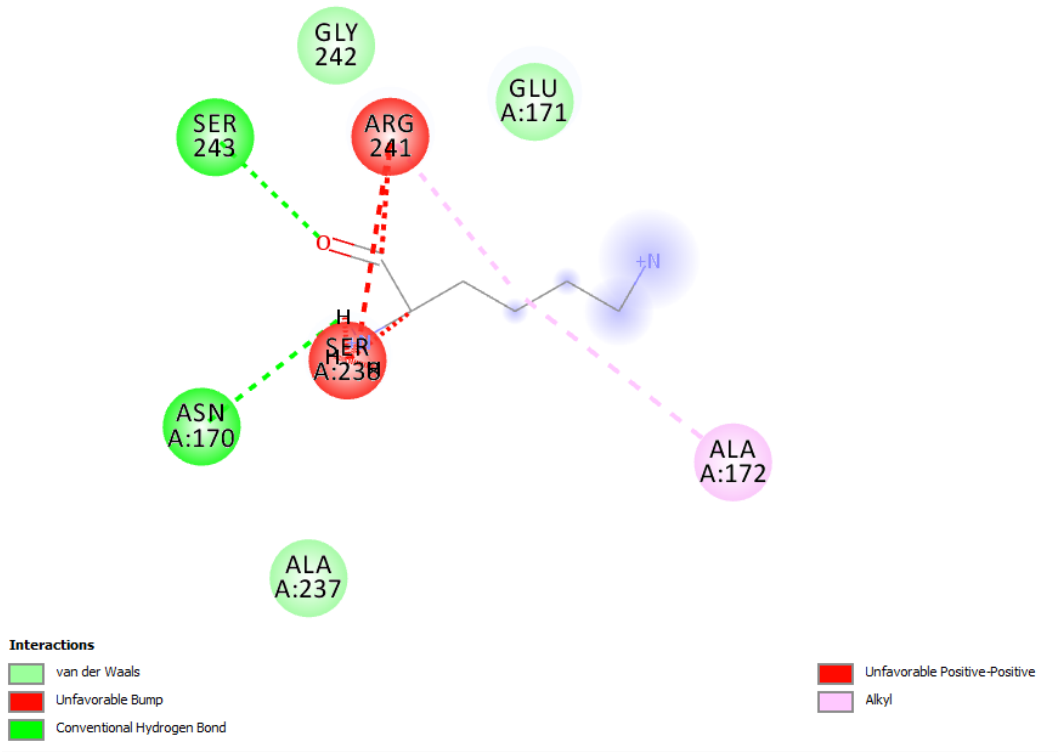

Figure 24: Interactions of cinnamyl acetate with extended-spectrum beta-lactamase

4.17 The Molecular Docking Studies of Linalool in Cinnamon with Extended-Spectrum Beta-Lactamase Protein in *Escherichia coli*.

The molecular docking studies of linalool with extended-spectrum beta-lactamase predicted the binding mode (Figure 25 and 26) which is evidenced by a fitness score of 38.03% in spite of the smaller molecular weight (196.29g/mol) of the ligand, linalool and the interactions were stabilized by van der waals interactions, conventional hydrogen bond, unfavorable bump, unfavorable positive-positive and alkyl interactions.

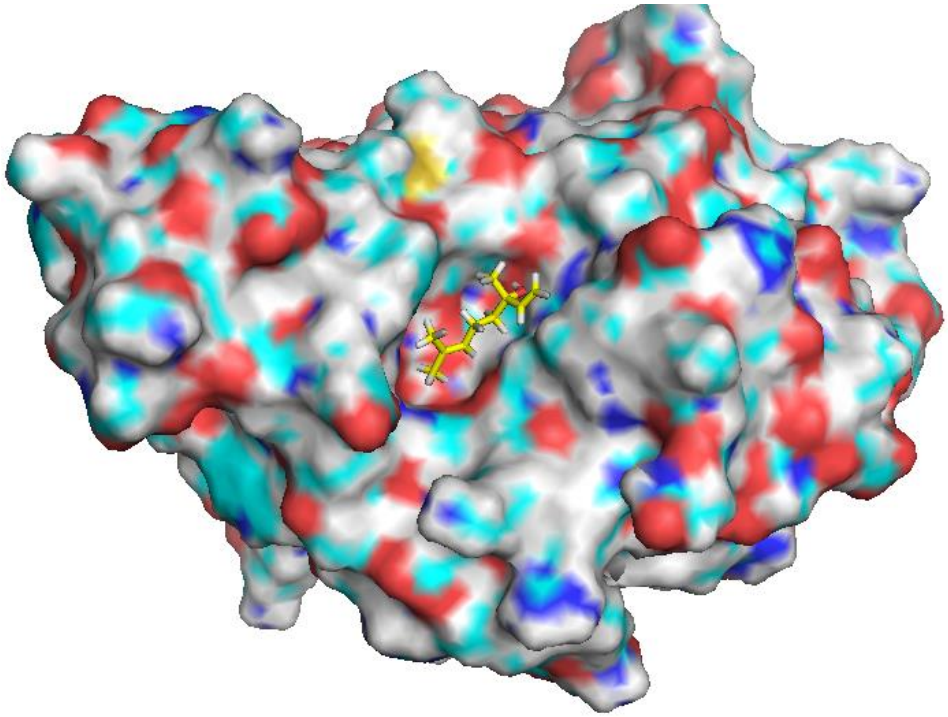

Figure 25: Visualized form of linalool-extended-spectrum beta-lactamase docked complex

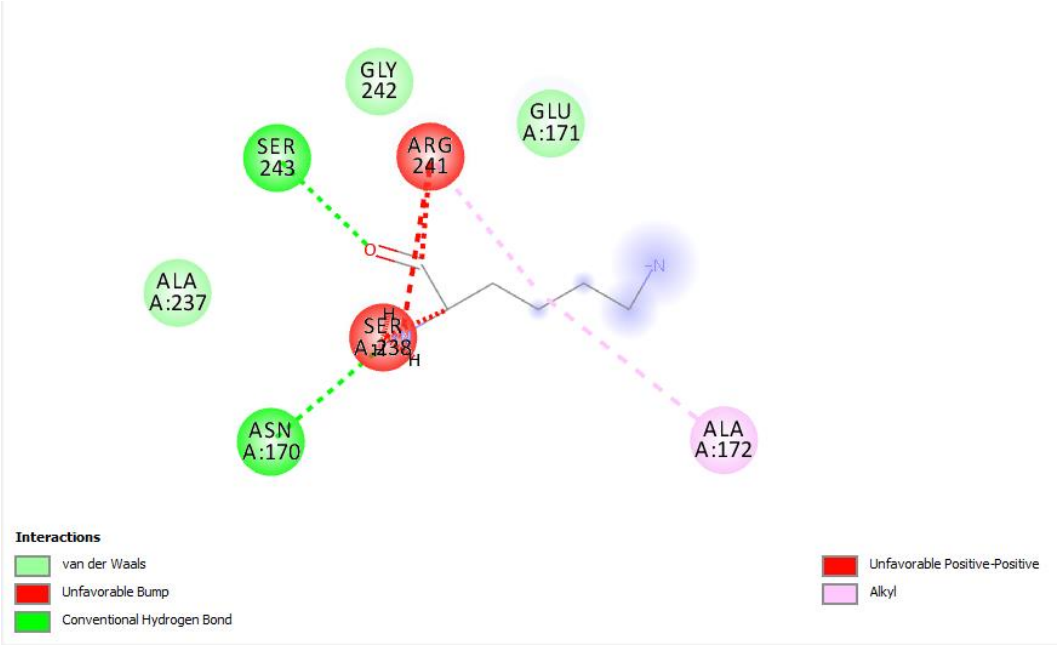

Figure 26: Interactions of linalool with extended-spectrum beta-lactamase.

4.18 The Inhibitory Effect of Ampicillin, Garlic Extract and the Combination of Ampicillin and Garlic Extract

The comparison of the zones of inhibitions of the ampicillin extract, garlic extract and the combination of ampicillin and garlic extract is presented in Figure 27. For the garlic extract only, the mean zones of inhibitions of 15 ± 0 mm, 15.25 ± 0.5 mm, 16 ± 2 mm, 17 ± 0 mm, and 18 ± 4 mm were obtained at concentrations of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, and 100mg/ml respectively and for the ampicillin only, zones of inhibitions of 13 ± 0 mm, 13.75 ± 0.5 mm, 14.25 ± 0.5 mm, 14.25 ± 0.5 mm, 14.5 ± 0 mm were obtained at concentrations of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, and 100mg/ml respectively whilst for the combination of garlic and ampicillin zones of inhibitions of 16.5 ± 3 mm, 18 ± 4 mm, 23.5 ± 1 mm, 27 ± 2 mm, 28.5 ± 7 mm were obtained at concentrations of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, and 100mg/ml respectively. The *Escherichia coli* was sensitive to the garlic extract and moderately sensitive to ampicillin extract but sensitive to the combination of the garlic and ampicillin.

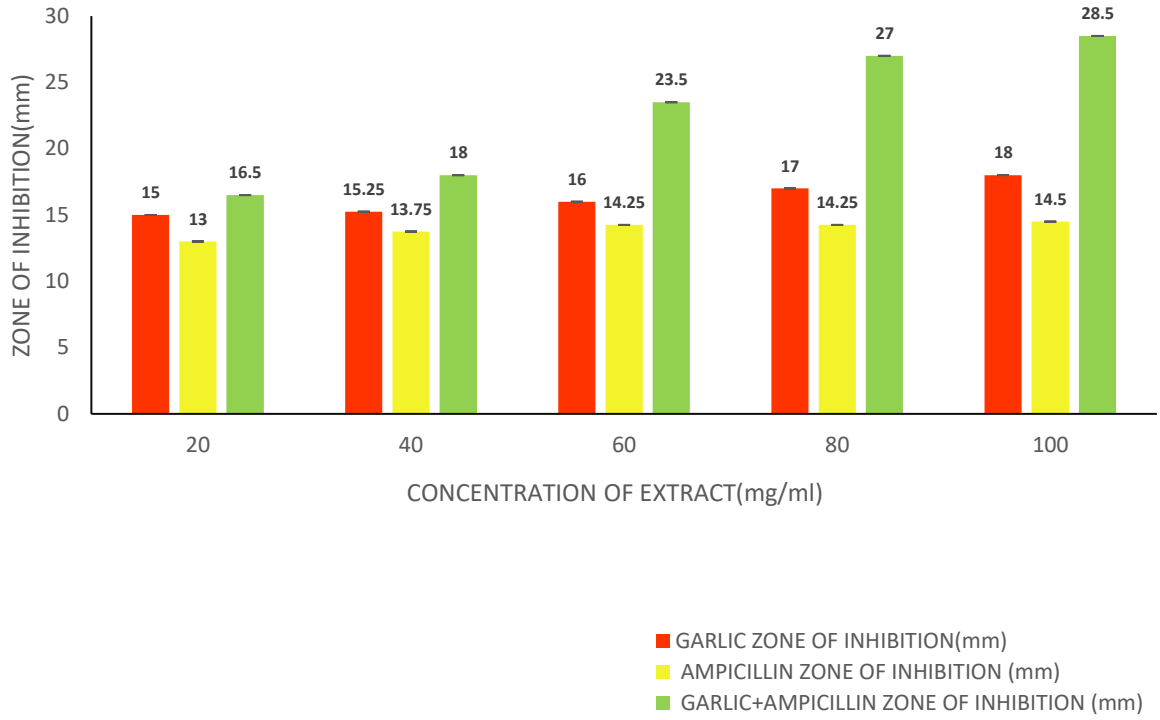

Figure 27: Mean zones of inhibitions of the ampicillin, garlic extract and the combination of the garlic extract and ampicillin at various concentrations.

CHAPTER FIVE

DISCUSSION

5.1 Phytochemical Screening of Ethanolic Extracts of Turmeric, Garlic and Cinnamon

In this study, flavonoids, saponins, alkaloids, coumarins and quinones were reported for turmeric, garlic and cinnamon. Tannins and oxalate were not detected in any of the three spices and this could be explained by the fact that the tannins and oxalate were destroyed by heat as reported by Khan (2003). Turmeric was the only spice that had terpenoids and cinnamon was also the only spice that had amino acids (Khare, 2021).

5.2 The Inhibitory Effect of Ampicillin, Turmeric Extract and a Combination of Turmeric Extract and Ampicillin on *Staphylococcus aureus*

S. aureus was sensitive to the turmeric extract only and the zones of inhibition were concentration-dependent. This can be compared to the work of Ramesh (2022) who observed that the flavonoids, curcuminoids, inhibits sortase A protein in *S. aureus* and since the phytochemical analysis of the turmeric extract reported the presence of flavonoid it can be concluded that the antimicrobial activity of the turmeric extract observed on the *S. aureus* is as a result of the phytochemicals like flavonoids which are present in the turmeric extract.

S. aureus was also sensitive to the combination of the ampicillin and the turmeric and the zones of inhibition were concentration-dependent. Also, the zones of inhibitions of the combination of ampicillin and turmeric were wider than the zones of inhibition of the ampicillin only and the turmeric extract only and this can be

compared to the work of Odunbaku (2008) who reported wider zones of inhibition for the combination of standard antibiotics and ethanolic extract of *Ficus exasperata* leaf on *S. aureus* where he obtained a minimum inhibitory concentration (MIC) of the plant extract on *S. aureus* at 100 mg/ml. Also, these wider zones of inhibitions obtained for the combination of ampicillin and turmeric can be compared to the work of Souto de Oliveira (2011) who investigated the synergistic activity of norfloxacin, tetracycline and erythromycin with ethanol extract of *Mangifera indica L*. peel against *S. aureus* strains. They observed that the individual extract did not display significant antibacterial activity (MIC ≥ 2048 mg/ml), but it modulated the activity of antibiotics (MIC = 512 mg/ml), that is, in combination with antibiotics, wider zones of inhibitions were observed. Moreover, since ampicillin was targeting penicillin binding protein (PBP) in *S. aureus* and turmeric was targeting sortase A in *S. aureus*, this multiple targets of both ampicillin and turmeric resulted in wider zones of inhibition for the combination of ampicillin and turmeric than the zones of inhibitions of the individual ampicillin only and turmeric only. Moreover, there were no clear significant difference between the zones of inhibition of the ampicillin, turmeric and a combination of the ampicillin and the turmeric at a concentration of 20mg/ml but as the concentration was increased from 20mg/ml to 100mg/ml, clear significant zones of inhibition were observed between the ampicillin, the turmeric and the combination of the ampicillin and turmeric.

5.3 Molecular Docking Analysis of the Ligands in Turmeric with Sortase A in *Staphylococcus aureus*

In order to gain better insight into the fitness, mode of interactions and binding efficiency of Sortase A protein in *S. aureus* with the ligands, (curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingeberene, curcumenol and curcumol) in turmeric, molecular docking was performed.

Even though the higher the fitness score the better the binding mode, the molecular weight of the ligand affects the fitness score hence with regards to small molecules, the fitness score is not expected to be higher to infer better binding mode or pose (Sharma, 2019). Thus, zingeberene, curmenol and curcumol had lower fitness score of 49.19%, 41.26% and 37.47% because they had lower molecular weight of 204.1878g/mol, 236.33g/mol and 236.35 g/mol respectively compared to curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin which had higher fitness score of 65.72%, 63.46%, 62.37%, and 61.80% because they had higher molecular weight of 368.38 g/mol, 308 g/mol, 338.4 g/mol and 372.4 g/mol respectively.

Thus, the molecular docking studies predicted the binding mode of curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingeberene, curcumenol and curcumol with Sortase A protein which is evidenced by a fitness score of 65.72%, 63.46%, 62.37%, 61.80%, 49.19%, 41.26% and 37.47% respectively. This agrees with the finding of Niu (2019), which reported that curcumin and its analogs inhibited sortase A through molecular docking and molecular modelling analysis. The fitness scores indicate that the curcumin,

bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingeberene, curcumenol and curcumol fitted into the active site of sortase A protein hence the ligands will easily bind to the sortase A protein to inhibit it in the *S. aureus* to stop it from anchoring virulence factors to the bacteria (Anjorin, 2013).

Moreover, the molecular docking predicted the binding efficiency of the ligands (curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingiberene, curcumenol and cucumonl) with the sortase A protein which is evidenced by the fitness scores. The binding efficiency is a measure of how strongly the ligands interact with the sortase A protein target. The higher the fitness score, the better the binding efficiency. Thus, curcumin, bisdemethoxycurcumin, demethoxycurcumin and tetrahydrocurcumin which had fitness scores of 65.72%, 63.46%, 62.37% and 61.80% respectively interacted strongly with the sortase A protein compared to zingiberene, curcumenol and curcumol which had lower fitness score of 49.19%, 41.26% and 37.47% respectively. The knowledge of the ligands (curcumin, bisdemethoxycurcumin, demethoxycurcumin and tetrahydrocurcumin) that interacted strongly with the sortase A protein could help in designing drugs that bind to their sortase A target in a specific and selective way.

The interaction of the curcumin with the Sortase A was stabilized by van der Waals interactions, conventional hydrogen bond interaction, carbon-hydrogen bond interaction, pi-pi stacked interactions, and alkyl and pi-alkyl interactions. The interaction of bisdemethoxycurcumin with sortase A was also stabilized by van der Waals and alkyl interactions whilst the interaction of demethoxycurcumin with sortase A was stabilized by van der Waals, conventional hydrogen bond, carbon-

hydrogen bond, alkyl and pi-alkyl interactions. Also, the interactions of tetrahydrocurcumin with sortase A were stabilized by van der Waals, conventional hydrogen bond, carbon-hydrogen bond, pi-cation, pi-sigma, alkyl, and pi-alkyl interactions whilst the interactions of zingiberene with sortase A was stabilized by van der Waals, alkyl and pi-alkyl interactions. Lastly, the interaction of curcumenol and curcumol with sortase A was stabilized by van der Waals and alkyl interactions.

The various interactions of the ligands with the sortase A protein play an important role in determining the function of the biological molecules and hence they revealed the inhibitory role of the curcumin, bisdemethoxycurcumin, demethoxycurcumin, tetrahydrocurcumin, zingiberene, curcumenol and curcumol to the sortase A in the docked complex. Also, the fitness score, binding efficiency and the interactions of the ligands with the sortase A residues give potent evidence of the efficacy of the ligands in turmeric in contributing to combating pathogenic Sortase A producing bacteria like *Staphylococcus aureus*.

Moreover, the binding mode, the binding efficiency and the various interactions predicted reveals that the ligands in turmeric binds perfectly with the sortase A protein in *S. aureus* and this reveals why the turmeric had antimicrobial effect on its own and also the zones of inhibitions heightened when the turmeric was combined with the ampicillin.

5.4 The Inhibitory Effect of Ampicillin, Cinnamon Extract and a Combination of Ampicillin and Cinnamon Extract on *Escherichia coli*

E. coli was moderately sensitive to the cinnamon and the zones of inhibition were concentration-dependent and this can be compared to the works of Yap (2015) and

Trombetta (2005) who reported that cinnamon extract has antimicrobial properties on E. *coli*. Moreover, it can also be compared to the works of Tung (2010) and Vangalapato (2012) who reported that cinnamon has the flavonoid, eugenol, which has antibacterial activity against *E. coli.* Since phytochemical analysis reported the presence of flavonoids in the cinnamon, it can be inferred that the antimicrobial property of the cinnamon on the *E. coli* is as a result of the flavonoids present in it.

E. coli was sensitive to the combination of ampicillin and cinnamon and the zones of inhibition were concentration-dependent. Moreover, the combination of the ampicillin and cinnamon gave wider zones of inhibition compared to the ampicillin only and the cinnamon only and this can be compared to the work of Purushotham (2010) who observed synergistic activity of tetracycline with methanolic extract of *Tectona grandis* against *E. coli.* Purushotham (2010) observed smaller zones of inhibitions for the tetracycline only and *T. grandis*, however, wider zones of inhibitions were observed for the combination of tetracycline and methonolic extract of *T. grandis* (62.5 mg/ml) against *E. coli* as the concentration increased. Moreover, from the works of Sauvage (2008) and Yap (2015), since ampicillin was targeting penicillin binding protein (PBP) in *E. coli* and cinnamon was targeting beta-lactamase in *E. coli*, this multiple targets of both ampicillin and cinnamon resulted in wider zones of inhibition for the combination of ampicillin and cinnamon than the zones of inhibitions of the individual ampicillin only and cinnamon only. It was also observed that there was no clear significant difference between the zones of inhibition of the ampicillin, cinnamon and a combination of the ampicillin and cinnamon at a concentration of 20mg/ml but as the concentration

was increased from 20mg/ml to 100mg/ml, clear significant zones of inhibition were observed between the ampicillin, cinnamon and the combination of the ampicillin and cinnamon.

5.5 Molecular Docking Studies of the Ligands in Cinnamon with Extended-Spectrum Beta-Lactamase in *Escherichia coli*

The eugenol, cinnamyl acetate, linalool, beta-aryophyllene and cinnamaldehyde in cinnamon were docked with the extended-spectrum beta-lactamase protein in *E. coli* to predict the fitness, mode of interaction and binding efficiency of these ligands with the protein.

Molecular docking studies of eugenol, cinnamyl acetate, linalool, beta-caryophyllene and cinnamaldehyde with extended-spectrum beta-lactamase predicted the binding mode which is evidenced by a fitness score of 40.64%,39.62%, 38.03%, 36.86% and 35.25% respectively and this confirms what Lena (2013) reported after docking the bioactive compounds in cinnamon, lower binding energies were observed which means the bioactive compounds in cinnamon had better interactions with the extended-spectrum beta-lactamase. Since eugenol, cinnamyl acetate, linalool, beta-caryophyllene and cinnamaldehyde are small molecules evidenced by a molecular weight of 164.2g/mol, 176.215g/mol, 196.29g/mol, 204.36g/mol and 132.16g/mol respectively, the fitness score was affected. Regardless of that, a fitness score of 40.64%, 39.62%, 38.03%, 36.86% and 35.25% for eugenol, cinnamyl acetate, linalool, beta-caryophyllene and cinnamaldehyde respectively predicted the binding mode of the various ligands with extended-spectrum beta-lactamase. The fitness scores reveal that eugenol,

cinnamyl acetate, linalool, beta-caryophyllene and cinnamaldehyde bound to the active site of the extended-spectrum beta-lactamase hence they will easily bind to the extended-spectrum beta-lactamase to inhibit it so that the extended-spectrum beta-lactamase in *E. coli* does not make the *E. coli* resistant to antibiotics administered to inhibit it.

Moreover, the molecular docking predicted the binding efficiency of the ligands (eugenol, cinnamyl acetate, linalool, beta-caryophyllene and cinnamaldehyde) with the extended-spectrum beta-lactamase protein which is evidenced by the fitness scores. The binding efficiency measures how strongly the ligands interact with the extended-spectrum beta-lactamase protein target. The higher the fitness score, the better the binding efficiency. Thus, eugenol which had a fitness score of 40.64% interacted strongly with the extended-spectrum beta-lactamase compared to cinnamyl acetate, linalool, beta-caryophyllene and cinnamaldehyde which had relatively lower fitness scores of 39.62%, 38.03%, 36.86% and 35.25% respectively. The knowledge of the ligand (eugenol) that interacted strongly with the extended-spectrum beta-lactamase protein could help in designing drugs that bind to the extended-spectrum beta-lactamase target in a specific and selective way.

Also, the interactions of the eugenol with the extended-spectrum beta-lactamase were stabilized by van der Waals interactions, conventional hydrogen bond interaction, and alkyl and pi-alkyl interactions whilst the interactions of cinnamaldehyde with extended-spectrum beta-lactamase was stabilized by van der Waals, conventional hydrogen bond, pi-cation, and pi-lone pair interactions. Also, the interactions of beta-caryophyllene, cinnamyl acetate, and linalool with

extended-spectrum beta-lactamase were stabilized by van der Waals, conventional hydrogen bond and, alkyl interactions. The various interactions revealed the inhibitory role of the eugenol, cinnamyl acetate, linalool, beta-caryophyllene, and cinnamaldehyde to the extended-spectrum beta-lactamase. Also, the fitness score, binding efficiency and the interactions show that the eugenol, cinnamyl acetate, linalool, beta-caryophyllene, and cinnamaldehyde in cinnamon have efficacy in contributing to the combating of pathogenic extended-spectrum beta-lactamase-producing bacteria like *E. coli*. Moreover, the binding mode, the binding efficiency and the various interactions predicted reveals that the ligands in cinnamon binds perfectly with the beta-lactamase protein in *E. coli* and this reveals why the cinnamon had antimicrobial effect on its own and the zones of inhibition heightened when the cinnamon was combined with the ampicillin.

5.6 The Inhibitory Effect of Ampicillin, Garlic Extract and a Combination of Ampicillin and Garlic Extract on *Escherichia coli*

E. coli was sensitive to the garlic extract only and the zones of inhibition was concentration-dependent and this can be compared to the work of Dashan (2017), who reported that garlic has the alkaloid, allicin, which is responsible for the antimicrobial property of garlic and since phytochemical analysis reported the presence of alkaloids, it can be inferred that the inhibitory effect of the garlic extract observed is as a result of the phytochemical presents in it.

E. coli was sensitive to the combination of ampicillin and garlic and the zones of inhibition was concentration-dependent. Also, the zones of inhibition of the combination of ampicillin and garlic were wider than that of the ampicillin only

and garlic extract only and this wider zones of inhibitions observed for the combination of ampicillin and garlic can be compared to the work of Adwan (2009) who evaluated the possible in vitro interaction between ethanolic extracts of *Rus coriaria* and certain known antimicrobial drugs including oxytetracycline and, penicillin G against clinical isolates of *E. coli* where wider zones of inhibitions were observed when the antimicrobial drugs were combined with the plant extract. Moreover, from the works of Sauvage (2008) and Dashun (2017), since ampicillin was targeting penicillin-binding protein (PBP) in *E. coli* and garlic was targeting DNA gyrase in *E. coli*, this multiple targets of both ampicillin and garlic resulted in wider zones of inhibition for the combination of ampicillin and garlic than the zones of inhibitions of the individual ampicillin only and garlic only. Also, there were no clear significant difference between the zones of inhibition of the ampicillin, garlic and a combination of the ampicillin and garlic at a concentration of 20mg/ml but as the concentration was increased from 20mg/ml to 100mg/ml, clear significant zones of inhibition were observed between the ampicillin, garlic and the combination of the ampicillin and garlic.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Phytochemical analysis reported the presence of various phytochemicals in the ethanolic extracts of garlic, turmeric and cinnamon. For the turmeric extracts, ampicillin and the combination of the turmeric extract and ampicillin, zones of inhibitions were observed for the various concentrations and the zones of inhibitions were concentration dependent but based on the Clinical and Laboratory Standards Institutes, the *S. aureus* was sensitive to the turmeric extract only but moderately sensitive to the ampicillin whilst for the combination of the turmeric extract and ampicillin, the *S. aureus* was sensitive to it with wider zones of inhibition than the individual turmeric extract only and ampicillin only. The molecular docking studies of curcumin, bisdemethoxycurcumin, curcumenol, curcumol, demethoxycurcumin, tetrahydrocurcumin and zingeberene in turmeric with the protein, sortase A in *S. aureus* predicted the binding mode which is evidenced by a fitness score of 65.72%, 63.46%, 41.26%, 37.47%, 62.37%, 61.80% and 49.19% respectively and the interactions were stabilized by the various bonds like hydrogen bonds, conventional hydrogen bonds, alkyl and pi-alkyl interactions etc.

For the cinnamon extract, ampicillin, and the combination of cinnamon extract and ampicillin, zones of inhibitions were observed for the various concentration and the zones of inhibitions were concentration-dependent but based on the clinical and Laboratory Standards Institute, the *Escherichia coli* was moderately sensitive to the

ampicillin and the cinnamon extract but sensitive to the combination of cinnamon extract and ampicillin with wider zones of inhibition than the individual cinnamon extract and ampicillin. The molecular docking studies of eugenol, cinnamaldehyde, beta-caryophyllene, cinnamnyl acetate and linalool with extended-spectrum beta-lactamase predicted the binding mode which is evidenced by a fitness score of 40.64%, 35.25%, 36.86%, 39.62% and 38.03% in spite of the smaller molecular weight of the ligands. The interactions of the various ligands with the extended-spectrum beta-lactamase protein were stabilized by van der Waals interactions, conventional hydrogen bond, alkyl and pi-alkyl interactions. For the garlic extract, ampicillin and the combination of garlic extract and ampicillin, zones of inhibitions were observed for the various concentrations and the zones of inhibitions were concentration dependent but based on the Clinical and Laboratory Standards Institute, the *Escherichia coli* was sensitive to the garlic extract and moderately sensitive to the ampicillin but sensitive to the combination of the garlic extract and ampicillin with zones of inhibition wider than the individual garlic extract only and ampicillin only.

6.2 Conclusions

Phytochemical analysis reported the presence of various phytochemicals in the ethanolic extracts. The ethanolic extracts had antimicrobial effect on the ampicillin-resistant bacteria and it was found that the combination of the ampicillin with the ethanolic extract of garlic, cinnamon and turmeric was more effective at stopping the growth of the *S. aureus* and *E. coli* as the concentration increased from 20mg/ml to 100mg/ml. That is, the combination of the ampicillin with the ethanolic extract

of garlic, cinnamon and turmeric would enhance antimicrobial activity by acting on multiple targets simultaneously, reducing the dose of the individual component and minimizing side effects.

Moreover, there were a clear difference between the zones of inhibition for the ethanolic extracts only, the standard antibiotic drug only and the combination of the ethanolic extracts and the standard antibiotic. That is, the differences in the zones of inhibition indicate the effectiveness of the antibiotic against the bacterial strain when the antibiotic is combined with the ethanolic extract, guiding clinicians in selecting the most suitable antibiotic combination therapy for individual patients.

Molecular docking studies of the various ligands in the ethanolic extracts with their protein targets in the ampicillin-resistant bacteria predicted an effective and better interactions which is evidenced by a good fitness score and the interactions were stabilized by the various bonds. Thus, the molecular docking of the ligands against their targets will make drug discovery faster, and cheaper, and further advances in docking procedures, combination with other computational methods, and new ways are expected, leading to more and better applications and easier drug discovery processes to help in developing new treatments.

The clinical implications of these findings are that it will help essentially for utilizing antibiotics safely and effectively, surveilling antimicrobial resistance trends, and informing public health measures. In the future, further research should be done by combining the spice extract with other standard antibiotics and its antimicrobial activity determined at higher concentrations. Moreover, the structural and functional projections of proteins by molecular docking techniques has the

ability to revolutionize future antibiotic drug discovery through a fast and inexpensive ways by optimizing antibiotic combinations for the treatment of bacteria that are resistant to antimicrobials.

6.3 Recommendations

1. Molecular interaction analysis should be done to predict the interaction and binding mode of the various ligands with their targets to help in drug discovery.
2. In the future, research efforts should be geared towards developing machine learning algorithms that can correctly predict molecular interactions to depict the interaction of antibiotic treatment combinations, as well as the interaction between antibiotics and their target bacterial proteins

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APPENDIX I

PLATES

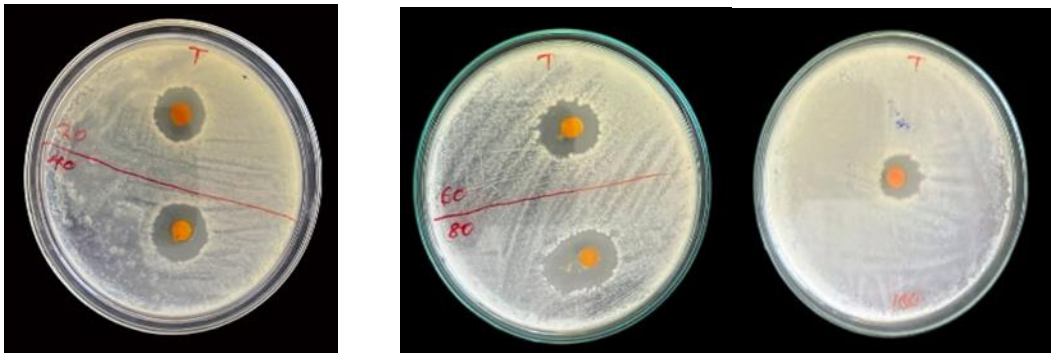

Plate 1A Plate 1B Plate 1C

Plate 1: Zones of inhibition of turmeric only on *Staphylococcus aureus*

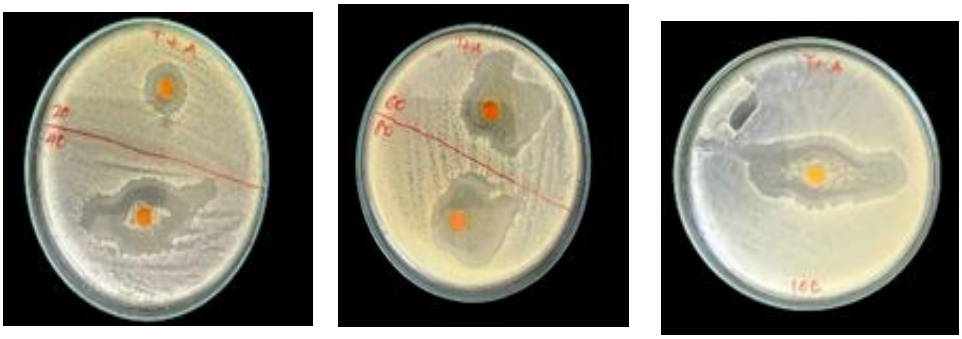

Plate 2A Plate 2B Plate 2C

Plate 2: Zones of inhibition of a combination of turmeric and ampicillin on *Staphylococcus aureus*

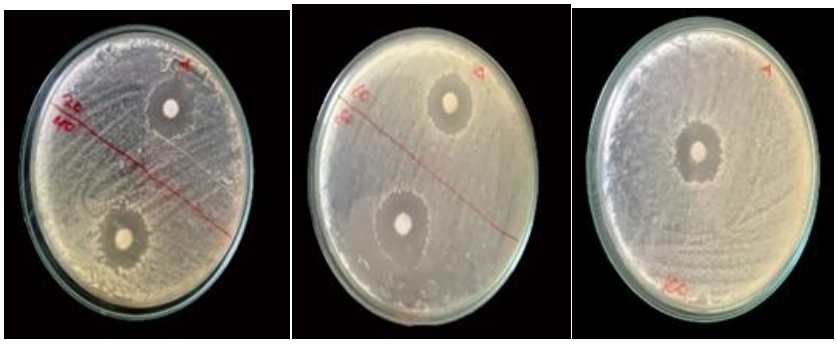

Plate 3A Plate 3B Plate 3C

Plate 3: Zones of inhibition of ampicillin on *Staphylococcus aureus*

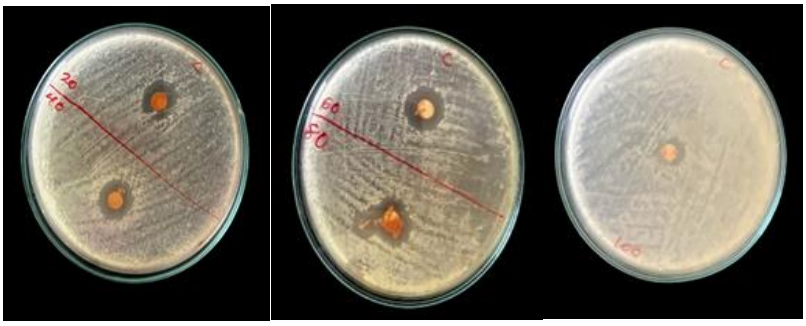

Plate 4A Plate 4B Plate 4C

Plate 4: Zones of inhibition of cinnamon only on *Escherichia coli*

Plate 5A Plate 5B Plate 5C

Plate 5: Zones of inhibition of combination of cinnamon and ampicillin on Escherichia coli.

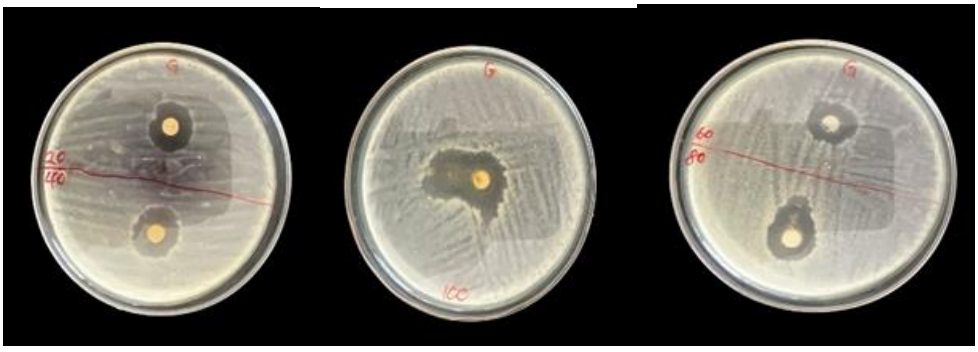

Plate 6 A Plate 6 B Plate 6C

Plate 6: Zones of inhibition of garlic only on *Escherichia coli*

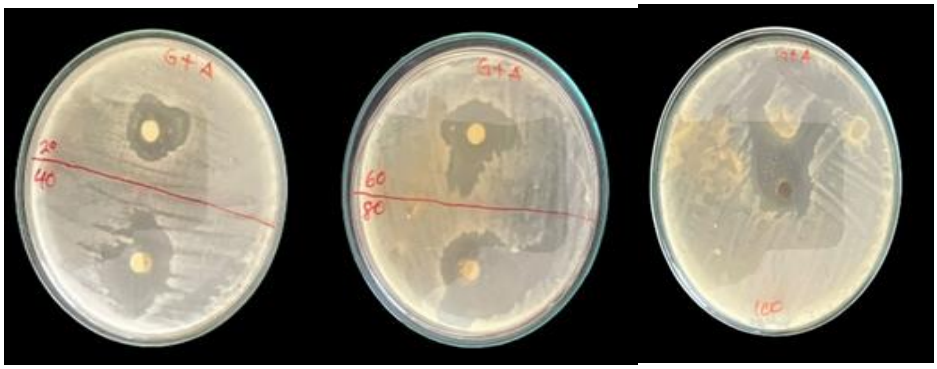

Plate 7A Plate 7B Plate 7C

Plate 7: Zones of inhibition of a combination of garlic and ampicillin on *Escherichia coli*

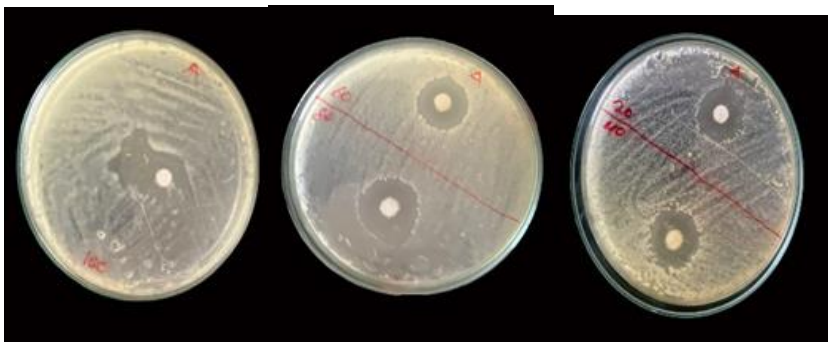

Plate 8A Plate 8B Plate 8C

Plate 8: Zones of inhibitions of ampicillin on *Escherichia coli*

APPENDIX II

ANOVA ANALYSIS

ANOVA Analysis of Ampicillin, Garlic, and a Combination of Ampicillin and Garlic

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONC. (mg/ml)	5	300	60	1000
AV OF GARLIC+AMPICILLIN ZONE (mm)	5	113.5	22.7	28.325
AV GARLIC ZONE (mm)	5	81.25	16.25	1.5625
AV AMPICILLIN ZONE (mm)	5	69.75	13.95	0.35625

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	6936.763	3	2312.254167	8.977503301	0.0010155
Within Groups	4120.975	16	257.5609375		
Total	11057.74	19			

ANOVA Analysis of Ampicillin, Cinnamon, and a Combination of Ampicillin and Cinnamon

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONC. OF EXTRACT (mg/ml)	5	300	60	1000
CINNAMON ZONE OF INHIBITION (mm)	5	65.5	13.1	1.58125
AMPICILLIN ZONE OF INHIBITION (mm)	5	69.75	13.95	0.35625
CINNAMON+AMPICILLIN ZONE OF INHIBITION (mm)	5	100	20	20.375

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	
Between Groups	7506.434	3	2502.145	9.790137	0.000661	3
Within Groups	4089.25	16	255.5781			
Total	11595.68	19				

ANOVA Analysis of Ampicillin, Turmeric, and a Combination of Ampicillin and Turmeric

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONC. OF EXTRACT (mg/ml)	5	300	60	1000
TUMERIC ZONE OF INHIBITION (mm)	5	85.75	17.15	1.675
AMPICILLIN ZONE OF INHIBITION (mm)	5	70.5	14.1	0.26875
TUMERIC+AMPICILLIN ZONE OF INHIBITION (mm)	5	130	26	46.25

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6660.209	3	2220.07	8.471983	0.001342	3.238872
Within Groups	4192.775	16	262.0484			
Total	10852.98	19				