ANTIBACTERIAL PROPERTIES OF TURMERIC ON SOME ENTERIC BACTERIA

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work was carried out by Eleojo Mercy AMEH in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

_____ _____

Dr (Mrs.) R. O. Okojie **Date**
(Supervisor)

APPROVAL

I certify that this work has been accepted in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc) in the Department of Microbiology, University of Benin, Benin City.

_____ _____

Prof. (Mrs.) F. I. AKINNIBOSUN **Date**
(Head of Department)

DEDICATION

This project is dedicated to God and my Parent Mr and Mrs D.O AMEH

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I want to use this medium to thank God Almighty for making this day possible for me. My sincere gratitude goes to my supervisor Dr. (Mrs.) R. O. Okojie, for her help and assistance during the duration of my project work. I also thank the Head of Department and other staff of the Department of Microbiology for their support.

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ABSTRACT

Turmeric belongs to the family of Zingiberaceae and natively grown in India and Southeast Asia. Turmeric plant contains rhizome which has several secondary metabolites including steroids, curcuminiods and sesquiterpenes with cucurmin being the principal component of the yellow pigment and the major bioactive substance curcumin. Enteric bacteria are Gram negative bacteria found in the human and animal intestine. Examples are *Shigella* sp, *Salmonella* sp and *Eschericha coli.* Antibacterial property of turmeric requires the use of its rhizomes. The rhizomes were shade dried and grounded to powder. 90g of the sample was measured and mixed with 150ml ethanol (ethanol extract), while 150g of powdered sample was mixed with 270ml of water (aqueous extract). Using Muller Hinton agar, cultures of test bacteria were swabbed on the Muller Hinton agar plates, a hole was drilled at the middle of the culture and then 0.01ml of the extracts was poured into the hole. The culture was incubated at 37^0C for 24 hours in an upright position. Results from both extracts (aqueous and ethanol) on test bacteria (*Eschericha coli, Salmonella* sp *and Shigella* sp) showed that turmeric has antibacterial property because it was able to Inhibit the growth of test bacteria. Ethanolic extract of turmeric (curcumin) showed more effects on enteric bacteria and it's because the ethanol helps to boost the antibacterial activities of turmeric than aqueous extract. Phytochemical screening was carried out on turmeric and it shows the presence of saponin, tannin, alkaloid, flavonoid and steroid chemical compound. The presence of curcumin inhibit the growth of enteric bacteria such as *Eschericha coli, Salmonella* sp and *Shigella* sp. Curcumin posses *invitro* antimicrobial potential against a wide range of microorganisms. Curcumin possesses a synergistic effect with important antibiotics such as cefixime, vancomycin and tetracycline against enteric bacteria. Turmeric destroys the bacteria cell membrane through penetration and therefore distortion of the cell shape happens as a result of exposure to curcumin. Thus, the damage of the cell membrane is the key mechanism of curcumin in enteric bacteria.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Turmeric is widely grown in tropical and subtropical areas and is mainly produced by India and other countries. Curcumin or diferuloylmethane is a major phytochemical of Curcuma longa L. (Zingiberaceae family), more commonly known as turmeric. Curcumin is a polyphenolic compound that gives the vegetable a yellow color. Traditionally, it has been used to enhance food, dye fabrics, and treat various ailments. Curcumin are extracted from turmeric by extracting solvent (preferably ethanol) through various methods (eg, Soxhlet, ultrasonic, microwave, and supercritical carbon dioxide) followed by purification by column chromatography (Sittiet *et al.,* 2009). Since curcumin was identified as a major component of turmeric, several pharmacological activities of curcumin including antimicrobial, antidiabetic, anti-inflammatory, anticancer, and antioxidant have been reported. Interestingly, when combined with other drugs, curcumin has been found to enhance the effects of antibacterial and antioxidant activities. Curcumin usually shows low or no toxic effects (Liang *et al.,* 2008). A systematic review from the online medicine computer database (Nirmala *et al.,* 2015) showed that curcumin is safe when taken up to 8 g each day for 3 consecutive months.

The antibacterial activity of curcumin was first demonstrated in nature in 1949. It was then discovered that turmeric some activities which include antibacterial, antiviral, anti-inflammatory, antioxidant, antiseptic and digestive activity (Nisar *et al.,* 2015). Phytochemical analysis of turmeric has revealed a large number of compound including Curcumin, volatile oil and curcuminiods which had been found to have potent pharmaceutical property (Bidwas *et al*., 2014).

The high *invivo* activity of curcumin was recorded against enteric Gram-positive (*Shigella* and *Yernisa*), other enteric Gram-negative bacteria (*E. coli* and *Klebsiella sp*). Studies had also highlighted that curcumin has a synergistic effect with important antibiotics such as cefixime, vancomycin and tetracycline against enteric bacteria (Chattopadhyay *et al.,* 2010). However, very few studies have shown the antibacterial activity of curcumin that appears to vary depending on the type of study. Studies have shown that the antibacterial activity of curcumin

against *Bacillus subtilis* occurs by inhibiting the proliferation of bacterial cells when inhibiting the assembly capacity that it has on the bacteria cell. However, it has an anti-infection function by affecting virulence, quorum sensing and biofilm activation. Moreover, these methods have not been proven in the case of other bacterial species, so they could not be generalized in all bacteria (Patra *et al.,* 2020).

Enteric bacteria are commonly found in the intestines of animals and humans. They may not be as harmful as the gut flora or microbiota, or can be pathogenic which means they cause infections. Usually, germs that enter the body enter through the mouth, usually through the consumption of food or water contaminated with animal or human feces. Other means of transmission include direct contact with contaminated water in swimming pools or ponds, contaminated areas or faeces of animals or humans that carry germs (Bhawana *et al.,* 2011). Human gut microbiota biotransform curcumin in a variety of ways including a series of spinal cord reduction of heptadienone and demethylation by *Blautia* sp. (Need *et al.,* 2015), and others to produce active metabolites that may have local or perhaps systemic effects. Curcumin and other curcuminoids have exhibited various medicinal functions; However, the full effect of curcumin on gut microbiota and the microbial metabolism of turmeric and related compounds are properly understood when more research were carried out (Need *et al*., 2015). In addition to this, curcumin has been labeled to be effective on anti-inflammatory and anti-infective (Hatcher *et al.,* 2008) and due to its abundant biological and medicinal properties is commonly referred to as "medicinal plant"(Rimple *et al*., 2012).

The specific symptoms of enteric bacteria infection vary depending on the type of bacteria involved and the specific area of infection. Common symptoms include fever, diarrhea, vomiting, and abdominal pain (Niamsa *et al.,* 2009). Treatment of people infected with pathogenic bacteria usually involves keeping them dehydrated and depending on the type of infection, considering antimicrobial treatment. Due to increased resistance to Gram-positive and Gram-negative bacteria, there are an urgent need to diagnose and test different antimicrobials, including those derived from plant substances with low human cytotoxicity (Wang *et al.,* 2009). Curcumin did not show any toxic effect on human health even when taken in doses of up to 8g per day. Understanding the mechanism of antimicrobial action Medicinal plant extracts are the first step in the effective use of these antibodies as a natural antibacterial agent. Based on the

available literature, there are two theories explaining the poly-pharmacological effects of curcumin (Cheng *et al.,* 2017). First, curcumin is known to be effective in many purposes, so it has various roles in regulating various cellular processes. Second, products caused by curcumin degradation have been shown to vary greatly depending on chemical or biological reactions. Many of these products were stable and effective in a different way that may lead to more results, and recent studies have reported that curcumin is effective against a wide range of drug-resistant strains of bacteria. To date, studies on the antibacterial activity of curcumin that exhibited antibacterial properties were increasingly being documented (Cheng *et al*., 2017).

Turmeric is an herbaceous perennial that reaches about three feet tall with grass-like leaves and greenish-yellow flows (Almayman *et al.*, 2013). Turmeric thrives incrainy tropical areas such as the Indian subcontinent and Southeast Asia (Sahal *et al*., 2009). Turmeric are often referred to as root but this is technically incorrect because turmeric rhizomes are underground stems that grow horizontally underground and have smaller roots growing from them (Velu *et al.,* 2017). The underground rhizome of turmeric consists of two distinct parts which are the mother rhizomes which is the extension of stem and the secondary rhizomes growing downward from the primary rhizomes.

The flesh of turmeric rhizomes are orange-brown, yellow or reddish-yellow in clolour. Ground dried turmeric are soft, fine and bright yellow in colour. Compound in turmeric called "curcuminiods" are responsible for the bright colours of turmeric. Turmeric has bitter, minty musty, medical and woody taste.

The name turmeric was derived from the Latin word *terra merita* (meritorious earth), referring to the color of ground turmeric, which resembles a mineral pigment. It is known as terre merite in French and simply as “yellow root” in many languages. In many cultures, its name is based on the Latin word *Curcuma*. Turmeric has 53 names around the world.

1.2 Aim and Objectives

The aim of this study was to evaluate the antibacterial properties of turmeric on some enteric bacteria. The objectives of this study were, to:

1. determine the antibacterial properties of tumeric.
2. determine the minimum inhibitory concentration.
3. determine bacteriocidal concentration of turmeric on some enteric bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview on Turmeric

Curcuma longa (Zingiberaceae) is a native plant of southern Asia and is widely grown in tropical and subtropical regions of the world. It is a member of the ginger family, derived from the ancient Arabic name for the kurkum plant known as saffron (Crocus sativus L.). The rhizome of the turmeric plant used as a spice, usually in dried form. However, in some parts of the far east, fresh turmeric is used and stored as ginger (Pulford 2003).

Turmeric is used as a remedy in Asian cuisine as a curry dish. It can also be added to chutneys, cucumbers and mustard in its color. Rhizomes contain 3-4% of flexible oils with different aromatic properties. Curcumin is the main active phytochemical compound of biological turmeric. Curcumin is the main curcuminoid of turmeric. The chemical formula for curcumin is $C_{12}H_{20}O_6$. It has a molecular weight of 368.38 g / mol. These curcuminoids are responsible for the yellow color of the root. In fact, it is the curcuminoids that contain all the bio-protective properties of turmeric (Badmaev et al., 2004). Turmeric has long been used as a powerful anti-inflammatory in both Chinese and Indian medical systems (Gescher et al., 2005). Turmeric has also been reported to have wound healing properties (Biswas and Mukherjee 2003). Turmeric are considered in some Asian countries as a dietary supplement, which is said to help with stomach problems and other ailments (Pulford 2003).

As a result of consumer misconceptions about food preservatives, the scientific community around the world shifting towards spices and herbs to use their antimicrobial properties to be used as natural food preservatives (Sagdic and Ozcan 2003). A large variety of these, including lemon grass and turmeric, have been shown to have antibacterial activity (antimicrobial activity) against foodborne microorganisms and foodborne illnesses (Grag and Menon 2003). However, information gaps still exist. In this study, the stem of lemongrass and leaves were examined separately; in addition, dried and fresh turmeric was also tested.

2.2. Chemical Components of Turmeric

Curcumin is a diferuloylmethane with a crystalline yellow-orange colour, molecular weight of 368.39 g/mol, melting temperature of 183°C and with the chemical formula $C_{12}H_{20}O_6$. Chemically, it exhibited keto-enol tautomerism, i.e it has a predominant keto form in neutral and acidic solutions, whereas the predominant form in the solid state and in an alkaline solution is its more stable in it's natural state (Anand *et al.,* 2007). There are two additional compounds known as curcumin which are curcumin [demethoxycurcumin1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione] and cucurmin [bisdemethoxycurcumin,1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione] (Buckingham *et al*., 2018).

2.2.1 Statement of Problem

Turmeric can make gallbladder problems worsen. There was some concern that turmeric can damage the liver, especially in people with liver disease, it is known to reduce blood clotting which may cause further bleeding during and after surgery. It was therefore advisable to discontinue the use of turmeric for at least 2 weeks before scheduled surgery (Koh *et al*., 2013). However, the activity of curcumin antibacterial has never been tested in clinical trials with the intention of using it as a future antibiotic. In addition, curcumin has been reported to block enzymes using drugs such as cytochrome (Sharma *et al.,* 2015). This could lead to the accumulation of unused drugs in the blood and cause unwanted toxins. In humans, however, the side effects of curcumin have been mild. Human studies have shown that curcumin ranging from 0.9 to 3.6 g per day for up to 4 months only causes certain side effects including nausea, diarrhea, and an increase in serum alkaline phosphatase and lactate dehydrogenase. In 2010, Balaji and Chempakam listed a few toxigenic and potent compounds derived from turmeric using a less expensive method of cheminformatics.

However, the selected compound should be tested in long-term studies on its effective dose against enteric bacteria to ensure the safety of using curcumin as a possible antibiotic. This could lead to the accumulation of non-metabolized drugs in the blood and caused unwanted toxicity (Tarcconie *et al.,* 2019). In humans, however, the adverse effects of curcumin have been relatively mild, showing that turmeric has led to chromosome disruption in the tested cell lines from 10 μ g / mL. In addition to the potential toxicity, poor digestion and low bioavailability,

curcumin faces many challenges when administered orally or intravenously due to the nature of the immune system.

2.2.2 Enhancement of the Bioavailability of Curcumin

Though curcumin has been considered as a promising antibacterial agent and has the potential to be used for clinical treatment, one of the major problems with ingesting curcumin by itself is its poor bioavailability, which appeared to be primarily due to poor absorption, rapid metabolism and rapid elimination. Enhancing the bioavailability of curcumin has received rather concerns in recent years. Using some agents to form a curcumin complex has been tested to improve the bioavailability of curcumin. For instance, piperine enhances the bioavailability of curcumin by 20 folds .Microemulsions of curcumin fabricated from food-grade ingredients, such as Tween 20, lecithin, vitamin E, and ethanol, increase the water dispersibility of curcumin by 1,000 to 10,000 folds. Nanocurcumin has also been considered as an alternative to improve the bioavailability of curcumin. Curcumin nanoparticle with the size of 2–40 nm exhibits more significant antimicrobial activity against *Shigella sp, E. coli* and *Salmonella sp*. Encapsulation of curcumin in liposomes enhances its water dispensability and increases its chemical stability, water dispensability and antioxidant and anti-inflammatory properties (Naira *et al*., 2019).

2.3. Curcuminoid

A curcuminoid is a linear diarylheptanoid, a relatively small class of plant secondary metabolites that includes curcumin, demethoxycurcumin and bisdemethoxycurcumin, all isolated from turmeric (curcuma longa). The values of turmeric on enteric bacteria are determined by curcuminoid content (Kulkarni *et al.,* 2012).

Although turmeric is thought to possess therapeutic properties, the curcuminoids complex comprises only 2.5-6% of the rhizome of tumeric of that, curcumin is approximately 77% of the curcuminoid content (Lee *et al*., 2013). To state this differently, it was understood that curcumin was the most abundant curcuminoid. It is also the most studied curcuminoid. Curcuminoids are only a small fraction of the turmeric rhizome and the rhizome is only one part of the plant. Unless explicitly stated that there was a different ratio of curcuminoids, it is assumed that the natural ratio of 77% curcumin, 17%, demethoxycurcumin and 3% bisdemethoxycurcumin with variances is maintained in a commercial product. However, curcuminoid has poor solubility in

water. According to (Ali *et al.,* 2015) curcuminoids solution in ethanol has absorption band in the region 240 -430nm which the maximum absorption was at 420nm.

In addition to these, inside a human body curcumin rapidly gets metabolized into many different end products. To name a few, hydrocurcumin (saturated forms like di-, tetra-, or hexa-hydrocurcumin), glucuronides, and sulfated curcumin (Anand *et al.,* 2008) moreso, initially, curcumin undergoes rapid intestinal metabolism to form curcumin glucuronide and curcumin sulfate via O-conjugation. Other metabolites formed include tetrahydrocurcumin, hexahydrocurcumin, and hexahydrocurcuminol via reduction . Curcumin may also undergo intensive second metabolism in the liver where the major metabolites were glucuronides of tetrahydrocurcumin and hexahydrocurcumin, with dihydroferulic acid and traces of ferulic acid as further metabolites . Hepatic metabolites were expected to be excreted in the bile (Shetty *et al*., 2009). Certain curcumin metabolites, such as tetrahydrocurcumin, retain anti-inflammatory and antioxidant properties. Apart from curcuminoids, *C. longa* is also the store house of bioactive compounds like ar-turmerone, turmerone, and curlone. In the past, they were neglected and were often considered to be contaminants of curcumin. Recent studies showed that even curcumin free turmeric extract is capable of eliciting biological activity hinting the presence of bioactives other than curcumin in it.

2.3.1 Physical, Chemical and Molecular Properties of Curcuminoids

Two active components of turmeric are the volatile oil and curcuminoids and both are present in oleoresin extracted from the turmeric root (Dune *et al*., 2019). The essential oils are composed mainly of sesquiterpenes, many of which were specific for the Curcuma genus. The aroma of this spice is principally derived from α- and β-turmerones and aromatic turmerone (Ar-turmerone). The chemical structures of curcuminoids $C_{21}H_{20}O_6$ makes them much less soluble in water at acidic and neutral pH, but soluble in methanol, ethanol, dimethyl sulfoxide and acetone. The curcuminoids give a yellow-orange coloration to turmeric powder due to the wide electronic delocalization inside the molecules that exhibit strong absorption between 420 to 430 nm in an organic solvent. The curcuminoids are mixture of curcumin, chemically a diferuloylmethane [1,7-bis(4- hydroxy-3-methoxy-phenyl)-hepta-1,6- diene-3,5-dione] mixed with its two derivatives, demethoxy curcumin [4- hydroxycinnamoyl-(4-hydroxy-3- methoxycinnamoyl) methane] and bis-demethoxy curcumin [bis-(4-hydroxy cinnamoyl) methane], defining the

chemical formulae as $C_{21}H_{20}O_6$ respectively. The Curcuminoids share the same structure with two benzenemethoxy rings, joined by an unsaturated chain. It has three important functions: an aromatic methoxy phenolic group; α,β-unsaturated β-diketo linker and keto- enol tautomerism. All these compounds exist in the trans-trans keto-enol form (Caichompo *et al.*, 1999). The aromatic groups provide hydrophobicity and the linker give flexibility. The tautomeric structures also influence the hydrophobicity and polarity. The hydrophobicity of curcuminoids makes them poorly soluble in water. Curcumin has a molecular weight of 368. 38g/mol and melting temperature of 183°C.

2.4. Antibacterial Activity of Turmeric

Bacterial infections are among the most important infectious diseases. Therefore, over 50 years of intensive research has been done to achieve new antiretroviral drugs. Despite advancement in the development of antibacterial agents, there was still a special need to find new antibacterial agents due to the development of drug-resistant pathogens (Wise *et al.,* 1998).

Curcumin has also been shown to have an *in vitro* anti-microbial potential against a wide range of fungal pathogens (Aggarwal *et al.,* 2103) and several Gram-positive and Gram-negative bacteria (Liang *et al.,* 2008; Rudrappa *et al.,* 2008). Recently, (Song *et al.,* 2012) showed that curcumin suppresses the attachment of Streptococcus mutants to the human tooth area and extra-cellular matrix protein. The study also highlighted that curcumin has a synergistic effect with important antibiotics such as cefixime, vancomycin and tetracycline against *Staphylococcus aureus* (S. aureus) (Betts *et al.,* 2009; Moghaddam *et al.,* 2009 ; Mun *et al.,* 2013)

(Tyagi *et al.,* 2015) The test of antibacterial activity of *Curcumin Ionga* against two Gram-positive bacteria (*S. aureus* & *E. faecalis*) and two Gram-negative bacteria (*E . coli* & *P. aeruginosa*) tested for killing assays using the ethanoic extract of turmeric and found that curcumin l was equally effective in all tested strains of bacteria from both Gram-positive and gram negative groups and the kill rate showed an increase in dose and incubation period.

A study by (Manjusha *et al*.,2014) in "Investigation of the Antibacterial Potential of Turmeric (*Curcuma Longa*) in enteric Pathogens" studies showed that 100% extract of turmeric water was highly antibacterial against *Proteus vulgaris*, *Staphylococcus aureus, Staphylococcus argenteus, Staphylococcus cornubiensis, Staphylococcus pneumoniae* (22-23mm zone of inhibition) while

at least antibacterial against *Pseudomonas aeruginosa*, *Enterobacter aerogenes* (10-11 inhibitors) . 50% aqueous extract of turmeric had anti-bacterial properties against *Proteus vulgaris* (21 mm barrier area) while at least antibacterial in *Salmonella typhi* (17 mm barrier area). 20% aqueous extraction of turmeric showed high anti-bacterial properties against *Proteus vulgaris, E.coli* 739 (18 mm barrier area) and at least against *Enterobacter aerogenes* (14-18 mm barrier area)

Also, (Manjusha *et al*.,2014) showed that ethanol extract of turmeric showed very high sensitivity against *Enterobacter aerogenes, E.coli* 739 (20 mm zone of inhibition) and low sensitivity to *Salmonella typhi, Proteus vulgaris, E.coli* 390 (18-mm blocking area). Ether emissions of turmeric showed very high sensitivity against *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhimurium* and low sensitivity against E.coli 390 (18-20 mm zone of inhibition). Methanol extract of turmeric showed very high sensitivity against *E.coli* 739, *E.coli* 390 (22 barrier area) and low sensitivity against *Salmonella typhi* (17 mm barrier area). Xylene extract of turmeric showed very high sensitivity against *E.coli* 739, (22 zone of inhibition) and low sensitivity against *Salmonella typhi* (15 mm zone of inhibition). Benzene extract of turmeric showed very high sensitivity against *E.coli* 739. (24 mm zone of inhibition) and at least antibacterial against *Salmonella typhi, Klebsiella pneumonia* (15 mm zone of inhibition). (Jacob *et al.,* 2014) Exfoliation of turmeric acetone showed a high degree of inhibition against *E.coli* 390 (22 mm barrier area) and low sensitivity against *Shigella*, *Salmonella typhi* (14 mm barrier area).

2.5 Enteric Bacteria

The intestines of all humans and animals are colonized by a large number of enteric bacteria. The majority of enteric bacteria are harmless and help maintain a healthy intestinal tract, and these are generally referred to as gut flora or human microbiota. However, other enteric bacteria are pathogenic causing illness. Most strains of *Escherichia coli* (*E. coli*) are harmless, but the pathogenic strains of *E.coli* produce toxins that may lead to foodborne illnesses with various outcomes. For example, *Escherichia coli* O157:H7 is an enterohemorrhagic *E.coli* (EHEC) that can cause bloody diarrhea in infected individuals (Peh *et al*., 2016). Conversely, enterotoxigenic *E.coli* (ETEC) can cause non-bloody diarrhea. E. *coli* is one of the most studied species of Enterobacteriaceae within the microbiota. Enterobacteriaceae, a large family of gram negative,

rod-shaped bacteria, is the enteric bacteria family most frequently studied in medical microbiology. In addition to playing a significant role in the intestines , Enterobacteriaceae may be found in the genitourinary tract and can, in some cases, cause urinary tract infections . *Shigella sp* , *Eschericha coli,* and *Salmonella sp* were also notable Enterobacteriaceae species. Other types of pathogenic enteric bacteria include *Salmonella , Proteus, Yersinia, Campylobacter jejuni* (*C. jejuni*), and *Clostridium difficile* (*C. diff*), which are gram positive bacteria. The *Shigella* sp species has many pathogenic subtypes including *Shigella boydii* (*S. boydii*), *Shigella dysenteriae* (*S. dysenteriae*), *Shigella flexneri* (*S. flexneri*) and *Shigella sonnei* (*S. sonnei*).

The main mechanism through which *E. coli* O157:H7, *Salmonella sp*, and *C. jejuni* enter the body is through contaminated consumable products, including unpasteurized milk, undercooked meat, foods prepared in unsanitary conditions, and contaminated drinking water. (Peh *et al*., 2016) *Shigella* can also enter the body through contaminated food and water, as well as through direct person-to-person or person-to-infected-animal contact. *Yersinia pestis* (*Y. pestis*) which is the cause of bubonic, septicemia may be transmitted to humans by small infected mammals (Todar *et al.,* 2020).

When pathogenic bacteria enter the body , they begin to multiply causing infection such as *Shigellosis* (caused by *Shigella* sp), Salmonellosis (caused by *Salmonella* sp). In response to infection, an individual's immune system works to eliminate the pathogenic bacteria. In most individuals, the immune system was able to fight off the infection, so illness does not result. However, when the immune system cannot clear the infection, cells in the body can become damaged, and an individual may experience signs and symptoms of illness (Frank *et al.,* 2015).

The level of *C. longa* supplementation as well as the presence of the pathogen could alter the pH of the duodenum. The increased in PH of *C. longa* supplementation facilitated bacterial proliferation to increase the diversity of the intestinal microbiota, particularly bacteria that are beneficial to the host (Huang *et al.*, 2008) reported that the low pH values in *Salmonella sp* infected chicks could be due to increased intestinal fermentation by *Salmonella* which produces volatile fatty acids. A high dose of Curcuma (3%) likely increased the prevalence of acidophilus bacteria (i.e., Lactobacillus) in this segment of the intestine. This prediction agrees with the results of (Lu *et al*.,2003), who reported that several factors might alter the pH and intestinal

microbiota, such as age, antimicrobial supplementation, and infection by pathogenic microbes. However, *C. longa* inhibit the growth of enteric bacterial.

2.6. Antibacterial Mechanism of Curcumin

2.6.1. Inhibition of the Bacterial Quorum Sensing (QS) System

Quorum sensing involves the process of interaction between microbial cells depends on the production and acquisition of chemical signals (self-inducers) to monitor cell density and complexity in human species. Quorum sensing (QS) is a molecular mechanism by which bacteria communicate to collectively adapt their behaviour according to cell density and the surrounding environment, QS allows bacteria to act as a cohesive group, by linking cohesive behaviors (Hawver *et al.,* 2016).

Biofilm refers to the fusion of tiny tissues wrapped in non-bacterial macromolecules. The formation of biofilms controlled by the QS system is a flexible process that combines bacterial adhesion, biofilm development, and maturation (Safadae *et at.*, 2015) Components of biofilms include various polymers, such as alginate, exopolysaccharides and proteins. Compared to planktonic growth, biofilms increase antibodies to antibiotics and were a major barrier to clinical treatment. Studies suggest that bacteria in biofilm are at a lower level of metabolism, and thus grow much slower than planktonic bacteria, making them more resistant to the effects of antibiotics.

In addition, were commonly found in gram negative bacteria due to the presence of extracellular polysaccharide matrix, biofilm resistance in bacterial biofilm in bacteria is 1000 times greater than in planktonic bacteria, which will also inhibit the effect of antibiotics on bacterial cells (Zhang *et al.,* 2019). Bacteria in biofilms are also protected by the interaction of immune cells. Therefore, the first step in eliminating pathogenic bacteria in biofilms is to prevent biofilm formation. Microbial cells in biofilms often require high doses of antimicrobials (Sharma *et al.,* 2014).

QS plays an important role in the maturation of bacterial films. Curcumin interacts with many cellular targets and transmission mechanisms through a multidrug-resistant infection strategy (Selvam *et al.,* 2014), in contrast to the single-agent approach currently used by most antibiotics

(Krausz *et al.,* 2014). Curcumin plays a preventive role through the bacterial QS system, not by killing the bacteria itself or by destroying the mature biofilm, but by making a protective effect on the biofilm formation process.

In addition, it reduces biofilm biomass, prevents adhesion and destroys biofilm formation by modeling the QS system. In addition, curcumin inhibits virulence-dependent features of QS (De Kievit *et al.,* 2001).

Curcumin has been shown to prevent the formation of biofilm by various bacteria in different parts of living organisms. The main biofilm filtration methods focus on directing the biofilm structure and assessing its volume, density, and similarity. (Izui *et al.,* 2016) found that testing for curcumin could prevent the formation of periodontal bacterial biofilms and reduce the number of bacteria. (Packiavathy *et al.,*2014) specifically identified curcumin-treated urinary tract pathogen biofilms and the number of microorganisms in biofilms using transparent microscopy and confocal laser-scanning confocal microscopy.

Additionally, they evaluated biofilm biomass quantitatively using a microtiter plate method to determine the effects of curcumin on the development of urinary pathogen biofilm. Their results showed a decrease in the intensity of biofilms in uropathogens treated with curcumin. In addition, curcumin has been shown to damage or release biofilm formation. In *Escherichia coli,* the biofilm thickness was reduced from 16 to 10μm, and in Proteus mirabilis, the control thickness was 11 μm and that the curofin-containing biofilm was 6.36μm. Similarly, Serratia marcescens also showed a significant decrease in biofilm density from 12 to 3.78μm (Delogu et al., 2013). However, curcumin has not been found to be effective in disrupting the matured biofilm of *Pseudomonas aeruginosa* (PAO1). Testing of the antibiotic activity of curcumin against uropathogens using a standard quantitative biofilm testing method reveals a decrease in the concentration of biofilm biomass of uropathogens when treated with curcumin. This suggested that the compound could influence the formation and density of biofilms in bacteria in order to achieve bacteriostatic effects.

(Gayani *et al.,* 2019) also demonstrated the antibiofilm effects of a natural curcuminoid-intercalated layered double hydroxide nanohybrid against *Staphylococcus aureus, P. aeruginosa,* and *Enterococcus faecalis*. Significant reductions in biofilm cell density and the amount of

extracellular polymeric substances (EPSs) were observed using scanning electron microscopy. The decrease in EPSs tended to reduce the amount of nutrients available for further cell growth and the conditioning surface for further attachment. Moreover, reductions in EPSs might prevent further biofilm formation because the EPS is a QS-dependent factor. Increasing the secretion of EPSs will led to changes in biofilm structure related to increased resistance to fungicides (Fulaz *et al.,* 2001). Therefore, inhibiting the production of EPSs would promote direct exposure of the microorganisms pathogen biofilm to antibiotics, thus promoting the removal of the biofilm. By observing the thickness and density of the cell membrane as well as changes in the outer polymers, researchers showed that curcumin could affect bacterial QS to destroy the structure of the biofilm, thus exerting bacteriostatic effects. (Gayani *et al.,* 2019).

Bacterial swimming and clustering behaviors are important toxic factors involved in the formation of biofilms. Previous studies have shown that curcumin can delay the formation of biofilms by keeping the cells in a planktonic state rather than in a biofilm state, which makes the cells more sensitive to the state of the biofilm (Sharma *et al.,* 2014). Curcumin treatment can also reduce urinary tract pathogen extracellular polysaccharide production, the production of alginate, and the swimming and clustering activities of QS, depending on various factors. For example, curcumin has anti-adhesion effects against Helicobacter pylori, suppresses all swimming of urinary tract pathogens, and blocks clustering behaviors when applied at relatively high concentrations, thereby inhibiting the migration of urinary tract pathogens. (Packiavathy *et al.,* 2014). The combination of antibiotics and nanoparticles has been shown to be effective in preventing bacterial biofilm formation (Yu *et al.,* 2020).

(Krausz *et al*., 2015) also found that curcumin nanoparticles inhibit the planktonic growth of gram-positive and gram-negative organisms and that the dose required to inhibit plankton formation was less than that required to inhibit biofilm formation. (Sharma *et al.,*2014) found not only that curcumin was effective against *Staphylococcus* epidermidis biofilm formation but also that its partial bacteriostatic concentration (FICI) value was reduced to half of that required for planktonic cultures. Therefore, curcumin could cause the formation of bacterial plankton, inhibited the migration of bacteria and reduce stability of the cell membrane. Curcumin can also inhibit virulence behaviors through the QS system. Virulence factors can be regulated by the signaling molecules of the QS system. For example, quinolone from *P. aeruginosa* and its

response regulator PqsR can act as a function of cell density to regulate various cell behaviors and modulate the production of virulence factors (Wu *et al.,* 2009).

Furthermore, the production of macropeptides by Martinella martensite depends on the independent behavior of the QS system, and the synthesis of those macropeptides is considered an important virulence factor (Liu *et al.,* 20009). Two signaling molecules, acylserine lactone and N-hexanoyl L homoserine lactone, have been shown to regulate the production of Lactobacillus (Morohoshi *et al.,* 2007).

In a study of the effects of curcumin on urinary tract pathogens, researchers showed that it reduced myricin production by inhibiting the production of megapeptide in Marcinella. Additionally, the effects of curcumin inhibition have reached 58%. In addition, the researchers showed that the anti-QS activity of curcumin was dependent on a focus on Viola cordierite pigment production value that the QS system relieved these problems. (Packiavathy *et al.,* 2014).

The antibiotic activity of curcumin was related to its autoxidation. By visual acuity of ultraviolet (UV), (Gayani *et al*., 2019) showed that compared with pure curcumin, product concentration and oxidation product types were significantly higher per unit time.

Furthermore, curcumin / lactate dehydrogenase (LDH) has a strong anti-bacterial activity against *P. aeruginosa, S. aureus,* and *E. coli* also demonstrated the activity of antibiofilm within 3 hours after treatment. These effects are related to the observation that the oxidation products of curcumin released by LDH disrupt bacteria after self-oxidation, leading to antibacterial activity. (Gayani *et al.*, 2019). Curcumin self-oxidizes in physiological pH and forms a spectrum of electrophilic and nucleophilic metabolites. A variety of previously unknown chemicals formed during autoxidation (quinone methide, peroxyl radical, endoperoxide, spiro epoxide, vinyl ether, and cyclopentadiene) are notable, and the identification of these molecules can establish a major paradigm of the novel when cellular curcumin, (i.e., polypharmacology) based on the number and variability of its automatic conversion products, can be understood (Gordon *et al.,* 2015).

However, no detailed studies have been conducted to determine the effects of these autoxidation products and the curmine intermediate, which is involved in regulating biological activity. In addition, further studies are needed to evaluate the roles of these compounds in the autoxidation mechanism of curcumin.

2.6.2. Effects on Deoxyribonucleic Acid

At genetic level, curcumin has been found to reduce the expression of bacterial gene expression, inhibit bacterial DNA damage (SOS response) and interact with DNA molecules to achieve bacteriostatic effects. In many bacteria the DNA is present as a single circular chromosome, although some bacteria may contain two chromosomes, and in some cases the DNA is linear rather than circular . In addition, curcumin down regulates the virulence factors in *P. aeruginosa* to activate biofilm-related genes (Rudrappa *et al.,* 2008). The bacteria itself has an SOS response where damaged DNA can be quickly identified and used to repair drug-induced DNA damage (Recacha *et al.,* 2019). Therefore, the SOS response is believed to contribute to cellular resistance, which is an important means of protection.(Zhang *et al.,* 2019) reviewed *Salmonella typhimurium* and *E. coli* by modulating the activity of β-galactosidase. They found out that curcumin inhibited the effects of UV radiation in a volume-dependent manner, acting as an effective SOS inhibitor and effectively preventing the re-activation of the phages. Compared with other bacteriostatic agents, curcumin works by a novel mechanism and prevents the development of drug resistance in cells.

RecA, an ATP-based protein, is involved in DNA repair and reunification, SOS response, horizontal gene transfer, movement, and biofilm formation. RecA also activates the SOS response and induces isolation of LexA inhibitors which may suppress the SOS response (Costa *et al.,* 2014; Gomez-Gomez *et al.,* 2007). Previous studies have shown that curcumin not only promotes the expression of the RecA protein in bacteria leading to a stronger response but also promotes apoptosis in *Eschericha coli* cells (Jenkins *et al.,* 2007).

Additionally, recent studied showed that curcumin is capable of self-oxidizing to form many intermediates (including free radicals and electrophilic intermediates), such as DNA and proteins necessary for bacterial proliferation, thereby inhibiting bacterial growth (Gayani et *al*., 2019). Accordingly, curcumin can inhibit bacterial Deoxyribonucleic acid (DNA) repair by producing partial intermediates and suppressing the development of drug resistance. However, to date, no reported studies have elucidated elucidated the intermediates or byproducts that affected bacterial cell DNA (Rao *et al.*,2020).

2.6.3. Effects on Proteins

Curcumin can prevent cell division by binding to microtubulins thus acting as an antibacterial agent, disrupting Ribonucleic acid (RNA) to disrupt protein synthesis and interact with proteins or enzymes. The heat-sensitive filamentous Z (FtsZ) is a prokaryotic homolog of the eukaryotic cytoskeletal protein tubulin that polymerize cells in the middle to form a z-ring, which then binds to bacterial cell division. The fusion dynamics of FtsZ protofilaments play a major role in the formation of the z-loop, and its integration and stability are important steps in bacterial cytokinesis. (Dipti *et al.,* 2008) found that curcumin inhibits the synthesis of FtsZ protofilaments and increases the activity of FtsZ glycoside triphosphatase (GTPase). Electron microscopic analyzes showed that curcumin reduced the accumulation of FtsZ protofilaments in vitro. Additionally, GTPase activity, which inhibited FtsZ synthesis, killed bacteria, and curcumin inhibits bacterial cell proliferation by blocking FtsZ binding kinetics in the z-loop. Recent research has shown that FtsZ may be an important target for anti-bacterial activity. In contrast, curcumin has been shown to disrupt protein synthesis by reducing RNA. (Mun *et al.,* 2014) found in their study of *S. aureus* that curcumin has significant effects on the levels of penicillin-binding protein 2a, encoded by the mecA gene.

In addition, curcumin often affect the secretion of cytoplasmic membranes and ABC carriers found in the cytoplasm of bacterial Gram-negative and Gram-positive cells (Poolman *et al.,* 2002). These vehicles have an ATP-dependent transport function, and the ATP enzyme inhibitor N, N'-dicyclohexylcarbodiimide may inhibit H + transport activity, thereby producing proton motility in the presence of compounds such as sodium azide (Jun *et al.,* 2012). Recent reports have shown that curcumin can be used to treat infection *S*. aureus by binding to ATP enzyme inhibitors (Mun *et al.,* 2014).

Moreso, curcumin inhibits the cellular protein sortaseA, thereby interrupting cell adhesion and interfering with biofilm formation (Coleman *et al.,* 2012). Similar to that with DNA, intermediates of the curcumin autoxidation system also interact with proteins to inhibit bacterial growth.(Li *et al.,* 2016) identified curcumin glucoside- and curcumin diglucoside-resistant strains of Streptococcus pneumoniae and showed that curcumin derivatives, particularly penicillin, exerts strong antimicrobial effects by inhibiting the penicillin-binding protein, and elicit destruction of the integrity of bacterial cell walls and disruption of the selectivity of

osmosis in the bacterial cell membrane, eventually killing the bacteria. (Mum et al.,2014) Thus, these derivatives might have various clinical applications in the treatment of pathogenic bacteria.

Curcumin directly interacts with cyclooxygenase 2, lipoxygenase (LOX), glycogen synthase kinase (GSK)-3β, phosphorylase-3 kinase, xanthine oxidase, Naminopeptidase, amyloid proteins, DNA polymerase, focal adhesion kinase, glutathione, pp60 SRC tyrosine kinase, thioredoxin reductase (TrxR), topoisomerase, ubiquitin different peptidase, and Toll-like receptor (TLR4). The binding constants of curcumin to these targets are often in the nanomolar range; for example, GSK-3β, 5-LOX, β-amyloid, and TLR4 have nanoscale binding constants, whereas glutathione S-transferase, TrxR, DNA polymerase-l, and tubulin have micromolar-scale binding constants (Aggarwal *et al.,* 2009). To improve our understanding of the bacteriostatic behaviors of curcumin, further studies are needed to assess whether the competitive binding of curcumin with these enzymes can affect the activities of bacterial cells.

2.6.4. Damage to the cell wall and cell membrane

Curcumin disrupts the localization of membrane-bound proteins and access to bacterial cell cells. Increased cell membrane permeability can improve bacterial sensitivity to other antibiotics. A study by (Barry *et al.,*2009) have shown that curcumin induced deep membranes in transbilayer orientation, while it is enhanced by hydrogen bonding in the phosphate group of lipids in a cholesterol-like manner. Like cholesterol, curcumin regulates phases in the membrane. By analyzing the dependence of the concentration of the order parameter found in NMR, the study also showed that curcumin forms oligomeric structures that are highly regulated in the elastic, and possibly thin, bilayer (Barry *et al*., 2009).

Curcumin promotes the formation of a highly curved hexagonal phase, which may contribute to exocytotic and membrane bonding processes within a cell. In addition, curcumin interacts with peptidoglycan to disrupt the integrity of the bacterial membrane, leading to cell lysis (Duracka *et al.*, 2019). Curcumin also increases access to the cell membrane in *S. aureus* and *E. coli*. (Tyagi *et al.,* 2015). *S. aureus* (MRSA) resistant methicillin untreated has been observed in electron microscopy transfer images of the complete diaphragm. However, cytoplasmic membrane damage was detected after curcumin treatment at 50% of the minimum inhibitory (MIC) concentration of 8 h; this is the same time effect seen in 100% of MRSA-

resistant methicillin cells. Changes observed in these cells include cell lysis, changes in ultrastructure, and cell fragmentation (Mun *et al.,* 2014).

In studies with *E. coli* ML-35p, curcumin and cinnamaldehyde have both been found to disrupt cell membranes, thus killing germs. Imaging MRSA cells in the presence of curcumin nanoparticles clearly demonstrated interactions between nanoparticles and cell membranes, leading to edema eruption and cell proliferation. Nanocarrier encapsulation makes free curcumin inaccessible to the surface of the pathogen to interact with the cell membrane, thereby enhancing the internal antibacterial properties of curcumin (Krausz *et al.,* 2017). In addition, curcumin exhibits significant antimicrobial activity when used in combination with various antibiotics in the subinhibitory dose of curcumin. (Tonon *et al.,* 2015) and increases bacterial sensitivity to β-lactam antibiotics, such as penicillin and methicillin (Mun *et al.,* 2014).

Studies on Gram-positive bacteria (*S. aureu*s) and Gram-negative bacteria (E. coli) have shown that differences in the external peptides of gram-positive bacteria and foreign phospholipids are negative bacteria that can lead to differentiation. of antimicrobial activities (Wamg *et al..,* 2017). According to a Gayani study, (2014) an increase in curcumin sensitivity was observed in the cell order *S. aureus*> *E. faecalis*> *P. aeruginosa.* The results showed that the sensitivity of gram-positive bacteria was greater than that of gram positive bacteria. These studies not only provided theoretical support for the prevention of different bacteria but also showed that their resistance was related to the formation and structure of their cell membranes.

Different effects of curcumin on gram-positive bacteria and gram-negative bacteria related to cell wall formation. The cell walls of many Gram positive cells consist mainly of several (up to 30) layers of glycans, each of which was connected by amino acid bridges to the other upper and lower layers. However, in gram negative bacteria, lipopolysaccharides are attached only to these two outer layers (Kokoulin *et al.,* 2020, Mun *et al.,* 2014) found that increased peptidoglycan concentration gradually inhibited curcumin activity, but did not cause complete inhibition, suggesting that curcumin may bind to cell wall and destroy its integrity.

2.6.5. Phototoxicity

Curcumin absorbs blue light (455–460 nm) in the absorption spectrum of 400–500 nm and can be used as an effective natural photosensitizer (Penha *et al.,* 2017) to promote the success of photodynamic processing. Gram positive bacteria are known to be more easily killed by anionic, cationic, and nonionic photosensitizers than gram-negative bacteria because gram-positive bacteria have a more porous structure in their cell membrane and are more easily penetrated by photosensitizers (Ghate *et al.,* 2019). More importantly, the cell walls of gram-positive bacteria are composed of a cytoplasmic membrane and a thick layer of peptidoglycan. According to a study by (Ghate *et al*., 2019) the attachment effect of lipid-soluble curcumin (hydrophobic) on peptidoglycan was poor. Thus, the Gram-positive cytoplasmic components of Lactobacillus monocytogenes might be vulnerable sites for curcumin-mediated photodynamic inactivation (PDI) with high inactivation efficiency.

Blue light activated curcumin does not exert bacteriostatic effects through direct contact with cells, but accomplishes this through the autoxidation mechanism of curcumin. These effects result in the production of intermediates and increase the amounts of oxygen free radicals in cells (Jiang *et al.,* 2017), thereby disrupting cellular integrity. Reactive oxygen species (ROS) have a short half-life; thus, contact between the photosensitizer and bacterial cells was important.

Indeed, the closer the photosensitizer is to the bacterial cell, the more likely there will be a negative effect of ROS on cell integrity (Gao *et al.,* 2020). Once bacterial cells are exposed to light, the photosensitizer absorbs the light energy, which is activated to produce ROS, such as hydrogen peroxide, superoxide, and singlet oxygen. ROS then oxidize components of cell membranes, including cholesterol, nitrogen- and sulfur-containing amino acid residues in proteins (Kim *et al.,* 2017), and guanosine in DNA and RNA, leading to bacterial death (Luksiene *et al.,* 2003) . Compared to that Gram positive bacteria, Gram-negative bacteria show stronger resistance to the phototoxicity of curcumin (Gao *et al.,* 2020).

(Huang *et al.*, 2020) showed that curcumin enhances antibacterial resistance of biofilms to blue light by detecting the DNA integrity, changes in protein expression and morphology, and virulence gene expression, providing a basis for the antibacterial mechanism. They showed that PDI (photodynamic inactivation) processing had a negative impact on the overall structure and

significantly reduced the adhesion ability of the biofilm in response to radiation. Moreover, analysis of the antibacterial mechanism in L. monocytogenes showed that the vulnerable targets of curcumin-mediated PDI were cytoplasmic DNA and proteins, rather than the extracellular membrane. In addition, PDI treatment was found to significantly downregulate virulence genes (e.g., inlA, hlyA, and plcA) in L. monocytogenes. The toxins expressed by these virulence genes determine the ability of L. monocytogenes to adhere, invade, and escape, thereby affecting its ability to cause infection. Curcumin plus radiation induces cell disturbance, cell degeneration, and cytoplasmic leakage; however, outer membrane rupture was not frequently observed.

(Hu *et al.,* 2018) suggested that photodynamic inactivation (PDI) requires three components which are light, a photosensitizer and oxygen. In this study, curcumin-mediated PDI treatment induced DNA damage and protein degradation; however, slight structural morphological deformations in the cells suggested that the DNA and proteins of Listeria monocytogenes cells were vulnerable targets for curcumin-mediated PDI. Notably, differences in cell wall structures between Gram-negative and Gram-positive bacteria are thought to be the main factors affecting the inactivation efficiency of PDI (Ghate *et al.,* 2015) because the main target of PDI is the external bacterial structure (Pereira *et al.,* 2014, Tonon *et al.,* 2015) combined curcumin and blue light to treat dental caries and confirmed that this treatment could inhibit the expression of population effect genes and formation of the biofilm, thus reducing the number of clinically isolated Streptococcus mutans and standard S. mutans bacteria.

Taken together, these findings show that the bacteriostatic mechanism of curcumin as a photosensitizer is similar to that without light activation. However, light-activated curcumin has been shown to improve targeting properties and could be used to increase drug targeting to specific tissues/organs (Bhattacharyya *et al.,* 2015).

2.6.6. Synergy

Synergy was generally thought to be limited to two antibiotics. However, recent research has shown that curcumin can be combined with traditional antibacterial agents and strong metals (Mansori *et al.,* 2019). It was synergistic with biological bacteriostatic agents and biological coupling agents and its antibacterial activity was higher than the sum of all individual components. Many studies on the synergistic effect of curcumin have compared the antibacterial

effect of curcumin when used alone with the antibacterial effect when used in synergy with other substances based on the MIC, bactericidal rate, cell uptake and other indicators (Raton *et al.*, 2011). Through synergistic effects, the dose of antibacterial activity can be reduced and different components can act on microorganisms in different ways, thereby interfering with the mechanisms of pathogen resistance (Deepika *et al.,* 2015). To date, curcumin has been found to function in synergy with various antibiotics or bacteriostatic agents, such as cephalosporin oxime, cefotaxime, vancomycin, tetracycline, ampicillin, evil Westwood, norfloxacin, ciprofloxacin, fish oil and chitosan and has been shown to synergistically enhance antibacterial activity against *S. aureus*. (Teow *et al.,* 2015 and Marathe *et al.,* 2013). Curcumin combined with ciprofloxacin was found to have antagonistic effects on Streptococcus typhoid. The antibacterial activities of curcumin and strongly bound metal complexes were enhanced by addition of the antibacterial agent cobalt nanocomposite or a silver nanocomposite film impregnated with curcumin (Hatamie *et al.,* 2012).

The synergistic effects of curcumin and cinnamaldehyde were found to not only be effective against the formation of *Staphylococcus* epidermidis biofilms but also reduced its FICI value by half (Sharma *et al.,* 2014). In the presence of curcumin, Bacillus subtilis showed increased sensitivity to echinacea*, E. coli* showed increased sensitivity to clindamycin, PAO1 showed increased sensitivity to azithromycin and Martensiella showed increased sensitivity to erythromycin (Packiavathy *et al.,* 2014). Curcumin in combination with nacetylcysteine, lactoferrin, and pantoprazole has been shown to substantially reduce symptoms of human H. pylori infection (Di Mario *et al.,* 2007).

Additionally, curcumin alone has antibacterial and anti-inflammatory activities and chitosan-polyvinyl alcohol at different concentrations has also been reported to exhibit antibacterial activities (Bano *et al.,* 2019). Notably, chitosan can enhance the polarity of the membrane and the combined use of curcumin and chitosan-polyvinyl alcohol has been shown to have synergistic effects on bacterial strains (Abbas *et al.,* 2019). Using trace dilution broth drug sensitivity testing and screening for dipeptides, fatty acids, and folic acid in Gram-positive bacteria and Gram-negative bacteria, it was shown that antimicrobial activity was 3.7- fold higher when curcumin was combined with these agent than when curcumin was used alone

(Singh *et al.,* 2010). These improved results might be related to similarities in the structures of these macromolecules with the bacterial cell wall, which contains amino acids and lipids.

Also, folic acid were co-factors for thymine and other nucleotides and ensured high cellular uptake of curcumin, thereby increasing the bioavailability of curcumin (Pal *et al.,* 2019). Based on these studies, we concluded that the main role of curcumin in synergy is to destroy the cell membrane or increase membrane polarity through its own bactericidal effects, resulting in increased sensitivity of bacterial cells to the synergistic substances.

The effects of curcumin vary depending on the type of bacteria. (Hashim *et al* 2019) showed that linseed oil and fish oil, which are rich in omega-3 fatty acids, could combine with curcumin, chitosan, and alginic acid salt to form composite beads. Treatment with fish oil-aluminum microspheres and curcumin fish oil-aluminum microspheres showed optimal bactericidal effects against gram-positive and gram-negative bacteria. Additionally, the incorporation of aluminum into curcumin and its composite beads improved the overall antibacterial activities against gram-negative bacteria, but reduced the antimicrobial activities against Gram-positive bacteria. Studies have also found that integration of these fatty acids into food preservatives inhibited bacterial growth, making them potential agents to improve food safety (Shin *et al.,* 2007).

A study using a mouse Candida infection model also showed the synergistic effects of curcumin with other components in terms of for reducing fungal loads in the kidneys of Swiss mice (Sharma *et al.,* 2010). The MIC values of curcumin plus ascorbic acid against different strains of Candida were also 5–10 fold lower than those that when curcumin was tested alone (Kdudsi Khalil *et al.,* 2012).

These synergistic effects indicated that curcumin and different fungicide materials can significantly stimulate synergistic activities and enhanced the antifungal properties of the compound (Jain *et al*., 2011). When curcumin as an antibacterial agent functions synergistically with other substances, it has the opposite antibacterial effect on gram positive bacteria and gram negative bacteria, compared to that when curcumin is used as an antibacterial agent alone. When used alone, gram positive bacteria are more sensitive to curcumin and gram negative bacteria are more sensitive when they are used synergistically (Banerjee *et al.*, 2014). However, the specific

reason which has not been elucidated as yet, might be related to curcumin's synergistic effect, playing a supporting role to improve the cellular uptake of other bacteriostatic agents rather than being the main bacteriostatic agent.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Sample Collection

Fresh rhizomes were bought from Oba market, Ring Road, Benin City. It was then shade dried for 3 days after which it was crushed into powder using blender. The sample was taken to the laboratory for experiment.

3.2 Extracts Preparation

A weight of 90g powdered sample was weighed in a weighing scale which was 150g and then the powdered sample was mixed with 270ml of water in a clean container and 60g of the sample was weighed and mixed with 150ml ethanol after which it was kept in a clean container. Both mixture was left for 24hrs and stored at interval. After leaving for the required time, samples was filtered using sieve and then finally with a whatman filter paper to prevent any residue giving it a smoother appearance, the ethanol mixture was first filtered before the aqueous mixture. Each filtrate was then placed in a beaker and left in a water bath at 50°C to concentrate. After proper concentration was done the samples were transferred to another clean container and kept in the refrigerator at 4°C till the time for analysis.

3. 3 Collection of Bacterial Strains

Different strains of bacteria were obtained at University of Benin Teaching Hospital (Microbiology Laboratory). After the collection of the strains, test were done for confirmation purpose after which it was kept in a clean container after it was labeled and kept in the refrigerator for preservation purpose. The bacteria used in the experiment were *Escherichia coli, Salmonella* sp and *Shigella* sp.

3.4 Antibiotic Susceptibility Test

Test organism was subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion on prepared media. Ten (10) different commercial antibiotic discs were used. Nutrient agar was prepared according to the manufacturer's instructions and boiled for proper mixture before it was autoclaved at 121°C for 15min, the agar was allowed to cool, it was poured in a sterile petri-dish and covered leaving it to solidify. The antibiotics that were used in this study was a negative antibiotic disc which contained Septrin (30µg), Chloramphenicol (30µg), Sparifloxacin (10µg), Ciprofloxacin (30µg), Amoxicillin (30µg), Augmentin (10 µg), Gentamycin (30µg), Pefloxacin (30µg), Tarivid (10µg) and Streptomycin (30µg). The antibiotic discs was carefully and firmly placed at the center on the inoculated plates using a sterile pair of forceps. The inoculated plates were inverted and incubated for 37°C for 24hours. The diameter of the zone of inhibition was measured in millimeters (mm) using a meter rule. The experiments was carried out in triplicates to minimize probability of error.

3.5 Nutrient Agar Preparation (NA)

Nutrient Agar medium was prepared by dissolving 28g of Nutrient Agar medium in 1000ml of distilled water according to manufacturer's instructions. It was mixed and the heated for it to dissolve completely for about 5min. Sterilize by autoclaving at 121°C for 15min. Then cool the agar medium at 45 - 50°C. Pour the agar into sterile petri-dish. It was poured inside the laminar air flow chamber to prevent contamination of the medium.

3.6 Muller Hinton agar (MHA)

Muller Hinton agar medium was prepared by dissolving 38g of Muller Hinton agar medium in 1000ml of distilled water according to the manufacturer's instructions. It was heated with frequent agitation and boiled to dissolve the medium completely at 100°C for 5min, sterilize by autoclaving at 121°C for 15min. It medium was allowed to cool at 45 - 50°C. Pour the agar into a sterile petri-dish. It was poured inside the laminar air flow chamber to prevent contamination of the medium.

3.7 Antibacterial Activity of Turmeric

Test isolates obtained from the University of Benin Teaching Hospital (Microbiology Laboratory) were used. The antibacterial activity against the pathogens was checked by agar well diffusion method. Cultures of pathogens were aseptically swabbed on Muller Hinton agar plates (standardized inoculums of the test bacteria adjusted to 0.5 MCFARLAND turbidity standards). Wells of 5 mm diameter was made aseptically by cork borer in the inoculated plates and different Concentrations was added into the labeled wells. The plates will be incubated at 37°C for 24 hour in upright position. The zone of inhibition in millimeter will be recorded with the help of meter rule. The experiments will be carried out in triplicates to minimize probability of error.

3.8 Minimum Inhibitory and Bacteriocidal Concentrations

1 ml of each sample at different Concentration was transferred to a test tube, 1 ml of nutrient broth will be added and then a loopful of the test organism previously diluted to 0.5 MCFARLAND turbidity standards was introduced to the tubes and turbidity after incubation.

3.9 Qualitative Phytochemical Screening

Test for Saponins

- 2g of powdered sample was boiled in 20ml of distilled water in a water bath and filtered.
- 10 ml of filtrate was mixed with 5 ml of distilled water and shake vigorously for a stable froth.
- froth was mixed with 3 drops of olive oil and shake vigorously.

Observation

Observe if any emulsion or persistent formation.

Test for Tannin

- 10ml of 0.1% Ferric chloride was prepared and dissolved in 10ml of distilled water.
- 0.5g of the powdered sample was boiled in 20ml of distilled water and then filtered.
- 5ml of filtrate was weighted and add 3 drops of 0.1% Ferric chloride.

Observation

Observe if any formation of yellow colouration.

Test for Alkaloids

- 100ml of 1% HCL , dilute 1ml HCL in 99ml distilled water.
- Weigh 0.5g of sample
- Add 15ml of 1% HCL and gently stir
- Place on a steam bath for 10mins and filter.
- 1ml of filtrate add 2 drops of dragenodroff's reagent.

Observation

Observe if any formation of yellow or orange-yellow precipitate.

Test for flavonoids

- Weigh 0.3g of sample in 30ml distilled water and allow to extract for 2 hours and filter.
- 10ml of aquoeus extract, add 5ml of 10% Ammonia followed by conc. Sulphuric acid.

Observation

Appearance of yellow colouration which disappears on standing is observed.

Test for steroids

- Weigh 0.3g of sample in 20ml ethanol
- Allow the mixture to extract for 2 hours and filter.
- 5ml of ethanoic extract and add 2 ml glacial acetic acid followed by 2 ml of Conc. H_2SO_4 (Sulphuric acid).

Observation

Observe if any formation of green colour.

CHAPTER FOUR

RESULTS

The antibacterial properties of turmeric on some enteric bacteria was carried out and the results were recorded.

Table 1 shows results of antibacterial Inhibitory action of ethanol turmeric extract on the three test microgramisms (*Eschericha coli, Shigella* sp and *Salmonella* sp) at different concentration. It also showed results of turmeric antibacterial Inhibitory action with aqueous extract..

Table 2 shows antibiotics sensitivity, resistance and intermediate results of turmeric on test organism (*Eschericha coli, Shigella* sp and *Salmonella* sp).

Table 3 shows Minimum Inhibitory Concentration (MIC) of the turmeric on the test isolates.

Table 4 shows Minimum Bacteriocidal Concentration (MBC) of the turmeric on the test isolates.

Table 5 shows the results of qualitative screening for phytochemical on ethanol and aqueous extract used. It also showed that saponin, alkaloid, flavonoid, tannin and steroid were present in turmeric.

Table 1: Zones of Inhibition of Turmeric Extracts on Test Isolates

Bacteria	Ethanolic Extract		Aqueous Extract	
	Concentration (mg/ml)	**Zones of Inhibition (mm)**	**Concentration (mg/ml)**	**Zones of Inhibition (mm)**
Shigella sp	1000	5	1000	7
	500	4.3	500	4
	250	6.7	250	2
	125	5.7	125	0
Eschericha coli	1000	12	1000	5.7
	500	6	500	3
	250	4	250	2.7
	125	2	125	0
Salmonella sp	1000	7	1000	2
	500	4	500	2
	250	2	250	0
	125	2	125	0

Table 2: Results of Antibiotics Sensitivity Test

ISOLATES	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	RESISTANCE INDEX
Shigella sp	30(S)	22(S)	0(R)	30(S)	10(R)	30(S)	30(S)	15(I)	7(R)	28(S)	3
Eschericha coli	28(S)	16(I)	10(R)	26(S)	0(R)	18(S)	28(S)	20(S)	14(I)	26(I)	2
Salmonella sp	30(S)	10(R)	8(R)	14(I)	6(R)	20(S)	28(S)	0(R)	18(S)	30(S)	4

NB:

RESISTANT (R) = 0-10mm

INTERMEDIATE (I) = 11-16mm

SENSITIVITY (S) = 17mm and above.

Keys: Gram negative antibiotic disc was used, which were: SXT=Septrin, CH=Chloramphenicol, SP= Sparifloxacin, AM=Amoxicillin,AU=Augmentin, CN= Gentamycin, PEF= Pefloxacin , OFX=Tarivid and S=Streptomycin.

Table 3: Results of the Minimum Inhibitory Concentration (MIC) of Turmeric on Test Isolates

Test Organisms	Extracts	MIC(mg/ml)
Shigella sp	Ethanolic Extract	125
	Aqueous Extract	250
Eschericha coli	Ethanolic Extract	125
	Aqueous Extract	250
Salmonella *sp*	Ethanolic Extract	125
	Aqueous Extract	500

Table 4: Result of the Minimum Bacteriocidal Concentration (MBC) of turmeric on test isolates.

Extracts	Isolates	Concentration	Growth	Inference
Ethanolic Extract	*Shigella* sp	125	+	No Bactericidal activity
	Escherichia coli	125	+	No Bactericidal activity
	Salmonella sp	500	+	No Bactericidal activity
Aqueous	*Shigella* sp	500	+	No Bactericidal activity
	Escherichia coli	500	+	No Bactericidal activity
	Salmonella sp	1000	+	No Bactericidal activity

Table 5: Qualitative Screening for phytochemicals on Extracts

Parameters	Turmeric
Saponin	+
Alkaloid	+
Flavonoid	+
Tannin	+
Steroid	+

Key:

+ = Present

CHAPTER FIVE

DISCUSSION AND CONCLUSION

The results obtained shows that turmeric has antibacterial properties that can inhibit entericbacteria growth. This research showed the activities of turmeric which include antibacterial, antiviral, anti-inflammatory, antitumor, antioxidant, antiseptic, cardioprotective, hepatoprotective, nephroprotective, radioprotective, and digestive activities. In a standard form, turmeric contains moisture (>9%), curcumin (5–6.6%), extraneous matter (<0.5% by weight), mould (<3%), and volatile oils (<3.5%).Nutritional analysis showed that 100g of turmeric contains 390kcal, 10g total fat, 3g saturated fat, 0mg cholesterol, 0.2g calcium, 0.26g phosphorous, 10mg sodium, 2500mg potassium, 47.5mg iron, 0.9mg thiamine, 0.19mg riboflavin, 4.8mg niacin, 50mg ascorbic acid, 69.9 g total carbohydrates, 21g dietary fiber, 3g sugars, and 8g protein (Balakrishnan *et al*., 1995).

The antibacterial activity of aqueous extract of turmeric indicated that the plant extract was a little effective compare to that of ethanolic extract against the tested organisms used in table 1. The extract inhibited all organisms at a higher Concentration of 1000 mg/ml with the highest average zone of inhibition (7 mm) against *Shigella* sp at 1000 mg/ml, highest zone of inhibition at 1000 mg/ml (5.7 mm) against *Eschericha coli* and least average zone of inhibition (2 mm) against *Salmonella* sp at 1000 mg/ml. The minimum inhibitory concentration (MIC) results of this extract shows that *Shigella* sp had MIC of 250 mg/ml while *Escherichia coli* has MIC of 250 mg/ml and *Salmonella* sp gave MIC values of 500mg/ml as shown in table 3.

Table 1 also showed that ethanolic extract of turmeric was very effective against the test microorganisms used. The extract inhibited all the organisms with the highest average zone of inhibition (12 mm) against *Escherichia coil* at 100mg/ml, highest zone of inhibition (7 mm) against *Salmonella sp* at 1000mg/ml and least average zone of inhibition (6.7 mm) against *Shigella* sp at 250 mg/ml. The minimum inhibitory concentration (MIC) result in shows that *Shigella* sp had MIC of 125 mg/ml while *Salmonella* sp had MIC of 125 mg/ml and *Escherichia coli* had MIC values of 125 mg/ml. The result suggested that ethanol Turmeric extract was active against Gram-negative bacteria (micro-organism used in this experiment). It also showed that the ethanol extract was preferable used for effective result against enteric bacteria.

The antimicrobial susceptibility test is an essential technique used in Pharmacology to determine the efficacy of novel antimicrobial agents from biological extracts against microorganisms (Pauletto *et al.*, 2016). The negative antibiotic disc was used for this experiment because enteric bacteria are Gram negative in nature. According to table 2 results, the zone of inhibition of each disc was measured and recorded. The highest zone of inhibition on this disc were Septrin, Chloramphenicol, Ciprofloxacin, Amoxacillin, Gentamycin, pefloxacin and Streptomycin at 30µg while the lowest has 10µg which are Sparifloxacin, Augmentin and Tarivid.

Moreso, the zone of extracts were measured and compared with the zone of the antibiotics disc as shown in 4.1. It was observed that the antibiotics disc has more clearer and wider zone than extracts as a result of it's chemical modifications (Wright 2010). Test isolates were resistance to Augmentin.

Turmeric extracts showed absolutely no bacteriocidal activity after bacteriocidal test was done. It was observed that the extracts only inhibited the growth of bacteria but does not kill them completely, so therefore extracts of the experiment are known to be bacteriostatic in nature (Wright 2010).

Phytochemical screening carried out on the aqueous - ethanol extract of turmeric revealed the presence of reasonable amount of alkaloids, saponin, tannins, coumarin, flavonoids, steroids, as shown in table 5. These antibacterial inhibitory effects could be attributed to the presence of these secondary metabolites. Alkaloids have been known to be the largest groups of secondary metabolites in plants, which was claimed to have powerful effects on humans and could be used as pain killers. Tannin is present in low concentration in the Turmeric extract. However, the presence of tannin could be responsible for its use in the treatment of intestinal disorders like diarrhea and dysentery as also shown in the high inhibition zone diameter for *Shigella* sp. The presence of curcumin in turmeric are responsible for its use as a potent antioxidant, antiplatelet, antimicrobial properties and its use to reduce cholesterol level and inhibit cancer growth.

CONCLUSION

The antibacterial activities of turmeric extract against enteric bacteria was more active with ethanol extract than aqueous extract. The extracts activity increased with an increase in the dosage of Curcumin as well as time exposure. Curcumin has ability to penetrate the enteric bacteria cell membrane leading to cell destruction. The use of turmeric as a spice and as a household remedy has been known to be safe for centuries. The beneficial effects of turmeric were traditionally achieved through dietary consumption. Extracts of turmeric (aqueous and ethanol) showed to be active on *Eschericha coli, Shigella* sp and *Salmonella* sp as it Inhibited the growth of the microgramisms. The presence of cucurmin in turmeric maked it active on enteric bacteria.

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APPENDIX A

EQUIPMENTS

- Refrigerator.
- Incubator
- Autoclave
- Micropipette.
- Test tubes.
- Cotton wool.
- Masken tap.
- Measuring cylinder.
- Face mask.
- Hand glove
- Petrish dish
- Oven
- Ruler
- Test tube racker
- Innoculating wire loop
- Ethanol disinfectant
- Bursen burner
- Forcep
- Hole driller
- Electronic measuring scale
- Scissors.
- Filter paper.
- Funnel.
- Conical flask.
- Electronic blender.
- Laboratory coat.
- Water-bath.
- Foil paper.
- Maker.

APPENDIX B

REAGENTS

- Powder rhizome of turmeric.
- Distilled water.
- Ethanol.
- Ferric chloride ($FeCl_3$).
- Sulfuric acid (H_2SO_4 also known as tetraoxosulphate vi acid).
- Hydrochloric acid (HCl).
- Sodium hydroxide (NaOH).
- Olive oil.

APPENDIX C

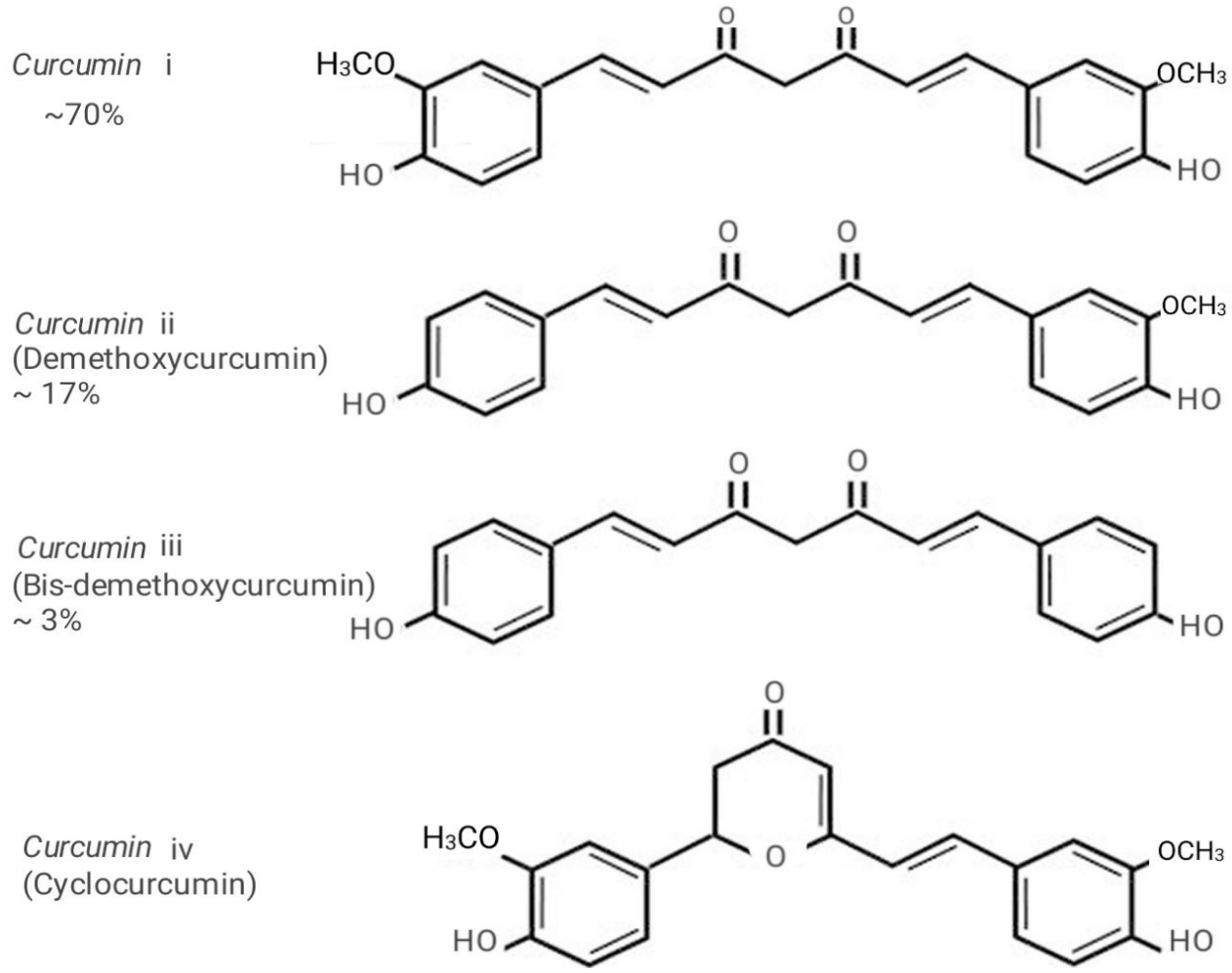

Fig 1: Structure of *Curcumin.*

APPENDIX D.

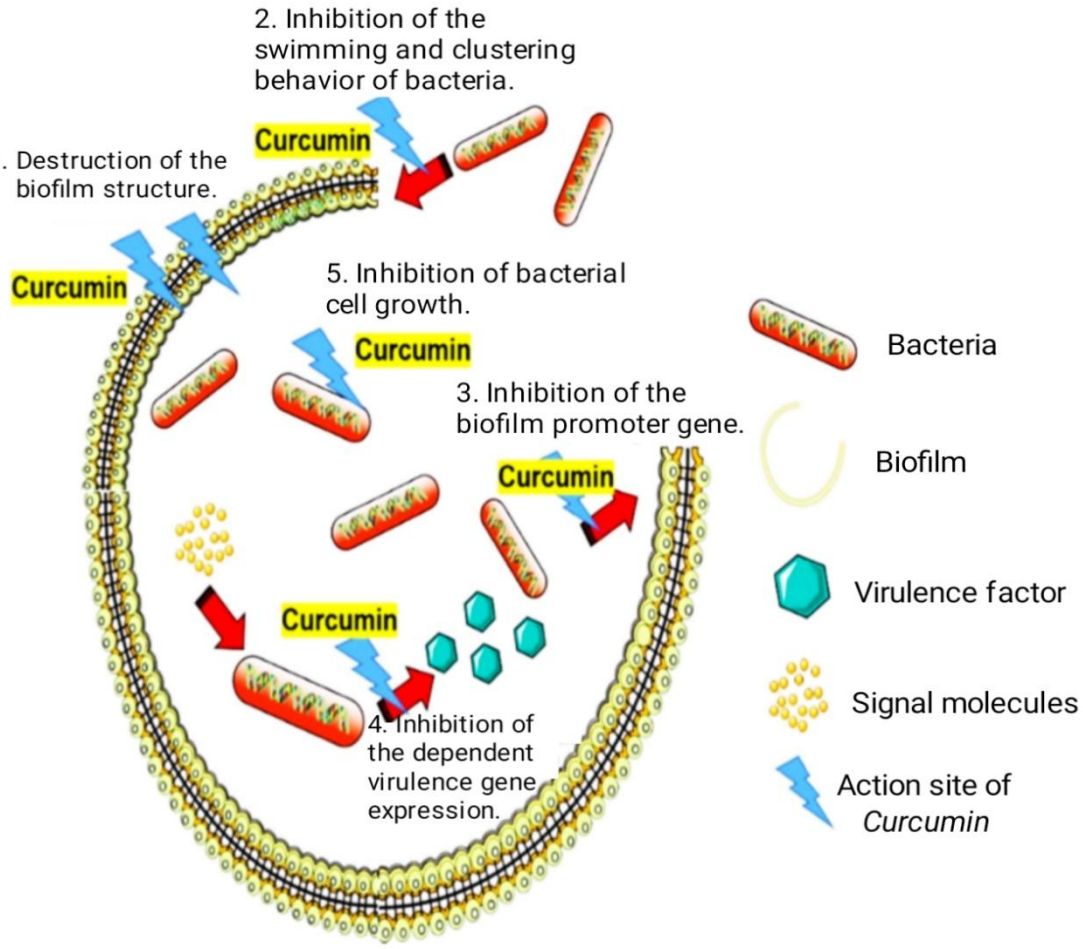

Fig 2: **Antibacterial mechanism of Turmeric.**