An-Najah National University

Faculty of Graduate Studies

CURCUMIN WITH SCHIFF BASE FUNCTIONALITY: SYNTHESIS, ANTIMICROBIAL ACTIVITIES

By

Rania Abed Allatef

Supervisor

Prof. Othman Hamed

This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Chemistry, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

2023

CURCUMIN WITH SCHIFF BASE FUNCTIONALITY: SYNTHESIS, ANTIMOCRBIAL ACTIVITIES

By

Rania Abed Allatef

This Thesis was Defended Successfully on 26/02/2023 and approved by

Prof. Othman Hamed	
Supervisor	Signature
Dr. Hisham Qarareya	
External Examiner	Signature
Dr.Ghaleb Adwan	
Internal Examiner	Signature

Dedication

To the soul of my father, may God have mercy on him

To my dear mother

To my brothers, sister and husband

To everyone who taught

Acknowledgements

Thanks to my God

Thanks to everyone who taught me something useful in my life.

I would like to express my heartfelt gratitude to my advisor professor Othman Hamed for his guidance, immense knowledge, support throughout

I would also like to thank my Co- Supervisor Dr. Hisham Qarareya Beside My advisors, I would like to express my deepest gratitude and appreciation to Prof. Ghaleb Adwan for his help and support during the Evaluation of antibacterial activity and genotoxicity of the prepared Curcumin-Based compounds.

Great thanks are extended to the teaching staff and to the technical staff in the laboratories in the department of Chemistry at An-Najah National University.

I'll be eternally grateful to my family, and doctors

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

CURCUMIN WITH SCHIFF BASE FUNCTIONALITY: SYNTHESIS, ANTIMOCRBIAL AND ANTIFUNGAL ACTIVITIES

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: Rania Abed Allatef

Signature: _____

Date: 26/02/2023

List of Contents

List of Tables

List of Figures

List of schemes

List of Appendices

CURCUMIN WITH SCHIFF BASE FUNCTIONALITY: SYNTHESIS, ANTIMOCRBIAL ACTIVITIES

By
Rania Abed Allatef
Supervisor
Prof. Othman Hamed

Abstract

Curcumin is a natural substance that has a wide range of biological functions and is a source to several medications. It is becoming more difficult to produce a natural-based drugs that can prevent bacterial antibiotic resistance. To achieve this goal, Schiff bases were prepared by a one pot process that involves reacting curcumin with various amino acids.

The amino acids selected for this work were those with heterocyclic functionality in addition to the amine and the carboxylic groups. The selected were histidine, lysine, cysteine, and arginine. The structures of the produced compounds were tested by FT-IR, H NMR, mass spectral analysis and antibacterial activity.

Schiff bases was tested against gram-positive bacteria which are Staphylococcus aureus, Staphylococcus epidermidis, and methicillin-resistant Staphylococcus aureus. And with gram-negative bacteria which are Escherichia coli and Klebsiella pneumonia.

The Schiff base 5 which produced from reacting curcumin with arginine was the most effective MIC value of 200 µg mL-1. The Schiff base 2 which produced from reacting curcumin with histidine changed the DNA of E. coli strain, this could be attributed to the presence of many nitrogen atoms in the structure of the compound.

Keywords: Schiff bases, natural substance, Curcumin, amino acids.

Chapter One
Introduction

1.1 Background

Natural remedies have been widely employed as folk medicine to treat a variety of illnesses that affected human health since the dawn of time. The use of bioactivity-guided isolation is a major focus of natural product discovery activities. Having obtained samples from a natural source the substance is examined for bioactivity. Compounds that, for instance, impede growth or trigger apoptosis are routinely found using cell-based assays. The crude natural product mixture's active ingredient is subsequently separated and given a structural description. However, in the majority of situations, nothing is understood about the precise process by which these natural compounds cause the bioactivity that is observed. The protein target(s) of each natural product must therefore be identified through mode of action investigations.

Compounds that do not function according to established pathways or mechanisms are frequently found in nature [1]. Despite the Sumerian civilization's 5,000-year-old written records regarding medicinal plants, archeological research has shown that the use of herbal medicines in Iraq extends back as far as 6000 years [2].

Any chemical components that have been obtained from or extracted from live organisms are considered natural products. To forecast the significance of transporters and enzymes in regulating drug bioavailability and disposition, the Classification System Biopharmaceutical Drug Disposition and Classification System (BDDCS) was proposed. Drugs are divided into one of four biopharmaceutical classes based on the degree of their metabolism and water solubility [3]. Although about 65% of the world's population relied primarily on plant-derived traditional medicines for their medical needs, plant products also play a significant, albeit more indirect, role in the health care systems of the remaining population, which is mainly concentrated in developed countries, but plants, animals (particularly spiders, insects, reptiles, and mammals), marine species, and microorganisms) can all be sources of biologically active natural compounds. Even though these active substances have been known to have biological activities and pharmacological potential, they are incredibly difficult to isolate (especially when the

substance of interest is not present at sufficient concentrations) and extremely difficult to synthesize [4,5].

As a complete organism like a bacterium or animal, or as a part of the organism like an animal organ or plant leaves, or as an isolated substance like coumarins, alkaloids, flavonoids, or steroids derived from the organism [6].

1.2 Categories of Natural products

Primary and secondary metabolites are the two basic classifications that can be used to broadly classify natural compounds. Nucleic acid, carbohydrates, proteins, and lipids are among the main metabolites.

These metabolites are necessary for an organism to survive. On the other hand, important intermediates in the basic metabolic pathways have been secondary metabolites. These metabolites may also appear briefly through the cycle of cells and may be exclusive to a certain class of organisms. Primary (or central) metabolism, which includes reactions and pathways absolutely necessary for survival, can be further divided into secondary (or specialized) metabolism, which carries out a variety of crucial functions for growth and development, including the interaction of the plant with its environment. Glycolysis, the TCA cycle, or the shikimate pathway's primary metabolic products frequently act as precursors for the production of the tens of thousands of secondary metabolites that have previously been identified. Secondary metabolism pathways exhibit a far higher degree of variation than main metabolism responses, which are substantially conserved at the level of species, organs, tissues, and cells, as well as during various developmental stages. Additionally, secondary metabolism's high level of catalytic promiscuity, which is most likely due to its recent divergence from primary metabolism and the weaker selection pressure applied to secondary metabolic enzymes compared to primary metabolic enzymes, is another factor that is presumably necessary for the large diversity of secondary metabolism. A recent metabolomics and statistical study comparing strawberries from wild and domesticated accessions revealed that domestication caused general dysregulation of secondary metabolism while the core primary metabolites were preserved, suggesting looser regulation of specialized metabolism. Domestication also caused general dysregulation of primary metabolism [7].

Common uses for secondary metabolites include defense against environmental stressors brought on by disease, insect, or herbivore attacks. In addition, numerous secondary metabolites might be capable of scavenging free radicals to help the organism deal with oxidative circumstances. Instead of primary metabolites, secondary metabolites are particularly referred to as "natural products" in medicinal chemistry [8,9].

1.3 Natural Products and Drug Discovery

Since the beginning of humankind's existence on Earth, natural products have played a significant part in therapy. Of all the ancient inventions, the art of using natural items to treat a variety of diseases is regarded as the most astounding development. Individual herbs or animal parts have been utilized as medicines throughout history by numerous cultures and groups to treat a variety of health issues. Only a few traditions advocated the use of potent combinations of several components in the form of poultices, tinctures, and mixes. The Mesopotamians were the first to use herbs like oils of cypress, cedar, liquor ice, myrrh, and poppy juice for treating different ailments in 2600 before century. Although the origin and chronology of the use of natural products in medicines are frequently disputed by scholars, it is recorded that all of these herbs are still used today to treat coughs, colds inflammation, and parasitic infections [10].

In all areas of medicine, including the creation of new drugs, there has been a significant advancement in science and technology. The search for secondary metabolites, their screening, and the creation of pharmaceuticals from natural materials are all difficult tasks that demand a lot of work. These include the following: identification of the plant material, collection, extraction, purification and structural characterization of the biologically active product, the last stage is the pharmacological and clinical evaluations. Natural products offer immense chemical variety and very unusual chemical structures that are greater than those provided by the other theoretical sources such as combinatorial and computational techniques, despite all of these obstacles. Modern medicinal chemistry has made significant strides thanks to technologies like high-performance liquid chromatography, nuclear magnetic resonance spectroscopy, mass spectrometry, microfluidics, and computer algorithms. This has made it possible to identify the chemical components of plants and use them to find new drugs. Many new drugs using plant-based natural compounds have been discovered as a result of tests using bioreactors and microfluidics technologies. Opium and morphine are a couple of these natural

remedies. In today's clinics and hospitals, a number of these substances' structural equivalents are utilized. Plant-based substances are showing promise as cancer treatments [11].

Only 27% of the medications currently on the market have purely synthetic origins, while roughly 73% of drugs identified in the last four decades come from natural compounds and their semi-synthetic counterparts. This provides the impression that natural based products remain the primary contributor to the creation of novel pharmaceuticals despite the substantial attention and resources directed toward combinatorial and synthetic-based drug discovery [12].

The class of natural products known as polyphenols appears to be one of the most appealing. Among these curcumin material is still one of the most investigated substances [13].

1.3.1 Antimicrobial Agents from Natural Sources

The majority of antibacterial and antifungal drugs available today have unwanted side effects, and intensive use of these drugs has sped up the evolution of germs that are resistant to common antibiotics, like methicillin-resistant Staphylococcus aureus (MRSA). The majority of commonly used antibiotics thus lose their efficacy in both clinical and agricultural settings. Novel, organically derived drugs are increasingly needed since they can prevent bacterial resistance and have fewer or no negative effects than currently available drugs. Curcumin will be covered in this search. Antibiotic drug resistance is a severe hazard to the general public's health. This is caused in part by improper use of antimicrobial drugs, antibiotics in animal and plant feed, poor hygiene habits, variations in the genetic make-up of microbes, and the function of free-living amoebas as genetic mixers for endosymbionts leading to increased resistance and virulence. Additionally, the rate of the appearance of resistance strains is far higher than the production of brand-new antibiotics. For example, multiple drug-resistant bacteria such as Methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus pneumoniae, Vancomycin-resistant Enterococci (VRE), Pseudomonas aeruginosa, Acinetobacter baumannii, Mycobacterium tuberculosis and Carbapenemresistant Enterobacteriaceae especially Escherichia coli and Klebsiella and Enterobacter are heralded as superbugs of the 21st century with an alarming mortality rate of up to 50%.

By 2050, it is predicted that up to 10 million people may die each year from illnesses that are resistant to treatment. If things don't change, we'll return to a time before antibiotics. There has been a 75% decrease in the number of newly approved antibiotics during the past 20 years. For instance, just three novel antibiotic classes—mupirocin in 1985, linezolid in 2000, and daptomycin in 2003—have been approved since 1970. Due to a lack of financial incentives, the pharmaceutical industry is producing fewer novel antibiotics, which has increased need for new medication sources from other industries. Finding new antibacterial chemicals is therefore urgently needed to address diseases caused by resistant bacteria in humans and animals.

The bulk of antimicrobials have been found in prokaryotes, however those that are eukaryotic in origin are mostly found in fungi and plants. Despite their remarkable ability to combat disease-causing organisms, the millions of animal species that live in a variety of habitats, from hot, desolate places to extremely cold ones, and those that eat polluted waste or rotten meat, have largely been overlooked as potential sources of antimicrobials. It goes without saying that they must have strong defenses against infectious diseases. Finding new medications to combat bacterial diseases with rising resistance will be aided by the discovery of novel antibiotic compounds originating from unexpected and natural sources.

Microbes are the source of the vast majority of antimicrobials. For instance, bacteria found in soil are the source of 70% of antibiotics that are sold commercially. Despite the fact that microbes coexist in a beneficial relationship, when nutrients are few, they can form an adversarial connection that leads to the production of substances that are poisonous to other species [14].

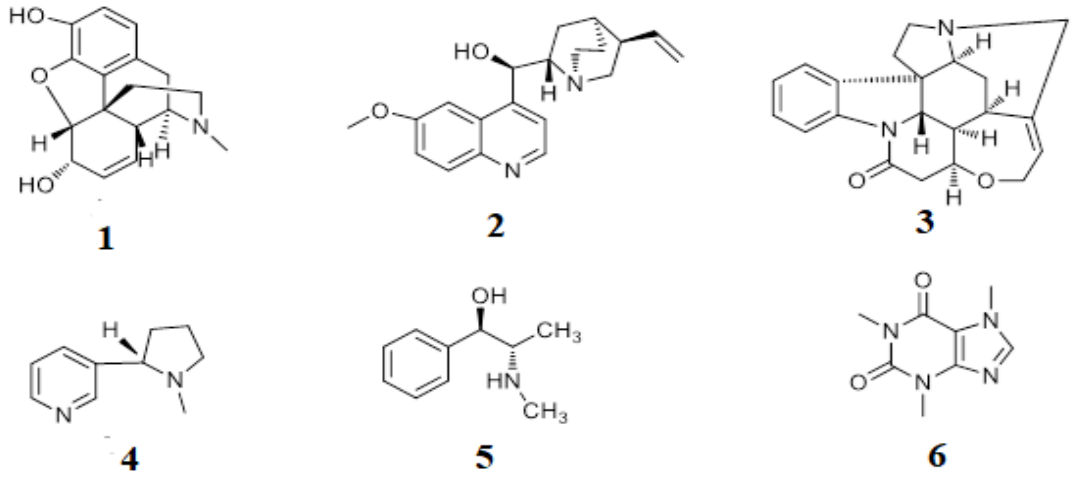
1.3.2 Natural Products Alkaloids

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. These nitrogen atoms cause alkalinity of these compounds. These nitrogen atoms are usually situated in some ring (cyclic) system. For example, indole alkaloids are those that contain nitrogen atom in indole ring system. Generally based on structures, alkaloids can be divided into classes like indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, and terpenoids and steroids. Other classification system is connected with a

family of plant species that they occur. One of the examples is the opium alkaloids that occur in the opium poppy Alkaloids affect humans and other animals in a variety of significant physiological ways. The most well-known alkaloids are shown in Figure 1.1, together with caffeine, nicotine, ephedrine, quinine, and strychnine.

Figure 1.1

Chemical Structures of morphine (1). quinine (2). strychnine (3). nicotine (4). ephedrine (5) and caffeine (6)



Similar to inorganic alkalis, alkaloids can react with acids to produce salts, hence the moniker "alkali-like" In acid-base processes, these nitrogen atoms have the ability to serve as bases. Alkaloids typically contain the suffix -ine in their names since they are considered amines and are named similarly. In their purest form, alkaloids are typically odorless, colorless crystalline solids, though occasionally they can also be yellowish liquids. They frequently taste harsh. Currently, 4000 different plant species include more than 3000 different alkaloids.

These substances are often produced by a wide variety of plant species, mostly by blooming plants and some animals. Numerous organic substances, commonly referred to as secondary metabolites, including amino acids, proteins, carbohydrates, lipids, and alkaloids are produced and stored by plants. They are kept in varying quantities in the leaves, stems, roots, and fruits of the plant. Despite previous claims to the contrary, evidence now points to the fact that they serve several crucial biological purposes in plants.

There are a few to as many as 30 different classes of structurally similar alkaloids found in plants. These alkaloids are members of the same class, but they differ in their structural makeup, with one typically making up the majority. Some plant families have a large number of alkaloids. There are roughly 30 distinct alkaloid kinds, for instance, in plants like the ergot fungus (Claviceps) and the opium poppy (Papaver somniferum). Their use in plants is still mostly unknown. Alkaloids are naturally occurring substances that discourage herbivorous creatures because of their bitter taste. They serve as natural insecticides on some plants. Alkaloids in plants may serve to shield them from some insect species' harmful behavior, according to a theory. Some animal species, including lizards, poison dart frogs (Phyllobates), and New World beavers (Castor canadensis), also contain alkaloids. Ergot, a type of fungus, also produces alkaloids.

Besides having the same general name—alkaloids—they have an extreme variety of chemical structures. Some of these compounds seem to have people known for ages because of their wide range of activity on human organisms and also other animals. For thousand years, extracts from plants containing alkaloids had medicinal use as drugs, and they owe their powerful effects thanks to the presence of alkaloids. Morphine was the first alkaloid which was isolated about 1804 from opium poppy in crystalline form. Alkaloids are an interesting group of compounds with a wide range of activities, undesirable and desirable, on animal and human organisms. Alkaloids have diverse physiological effects: antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and antitumor activity and many others. Nowadays, alkaloids usually from plants rather than from animals are still of great interest to organic chemists, biologists, biochemists, pharmacologists, and pharmacists. Well-known alkaloids include morphine, strychnine, quinine, atropine, caffeine, ephedrine, and nicotine.

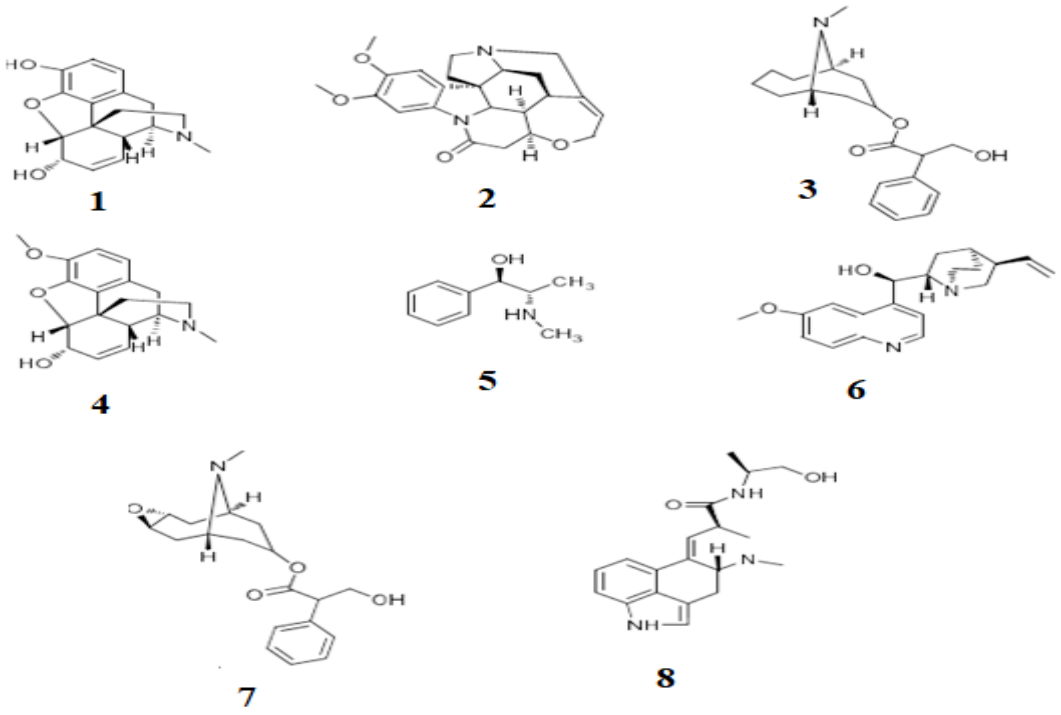
People have known and used plant extracts with alkaloids due to their wide range of activities for centuries. However, direct techniques to isolate pure chemicals from certain plant species were not known back then. Plant tissues typically contain aqueous solutions of alkaloids. The extraction technique is typically used to isolate them. Special extraction techniques for alkaloids with economic value were created. Alkaloid-containing mixtures should typically be dissolved using a solvent and reagents. Alkaloids in the solution can be recovered using the extraction method. Each alkaloid can then be extracted in pure

form by being separated from the mixture. Certain solvents must be employed in order to get alkaloids in their crystalline form. Chromatography is another approach. It makes use of variations in the rates at which various alkaloids in a solvent system adsorb on solid substances like silica or alumina.

Because they have a proton donor groups and one or more proton acceptor such as N in their chemical structures, alkaloids easily establish hydrogen bonds with enzymes, proteins, and receptors. The alkaloids' greater bioactivity is explained by their possession of functional groups that may donate and accept protons, such as polycyclic moieties and phenolic hydroxyl. Analgesic (codeine), antihypertensive (ephedrine), anticholinergic (atropine), antipyretic (quinine), antihypertensive (reserpine), antiemetic (scopolamine), antitumor (vinblastine), antimalarial (quinine) and vasoconstrictor oxytocic (e.g. ergometrine), activities are among the pharmacological properties. The structure of the aforementioned alkaloids is depicted in Figure 1.2 [15].

Figure 1.2

Chemical Structures of morphine (1), brucine (2), atropine (3), codeine (4), ephedrine (5), quinine (6), scopolamine (7), ergometrine (8)

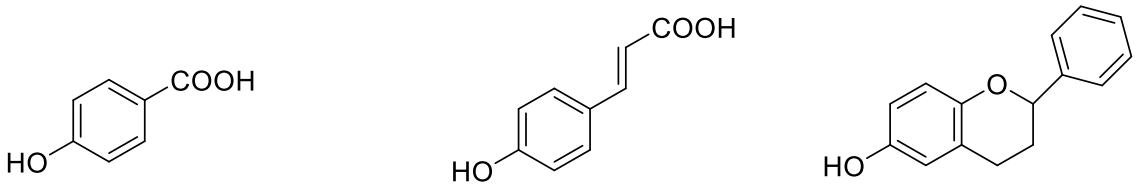


1.3.3 Phenolic From Natural Sources

Common phytochemicals called phenolic compounds are present in all plants. Examples are shown in Figure 1.3, they have an aryl ring with one or multi O-H functional groups. Phenolic substances are divided into simple phenols and polyphenols according to the quantity of aryl units present in the chemical structure of the molecule. Examples on the phenolics are phenols, lignans, lignin, phenolic acids, coumarins, condensed tannins, flavonoids, curcumin and hydrolysable tannins are examples of plant phenolics. Phenolics function as antioxidants that lower inflammation, reduce the risk of diabetes, prevent heart disease and cancer, and lower mutagenesis rates in human body cells [16,17].

Figure 1.3

Chemical structures of flavonoids and phenolic acids



Hydroxybenzoic acid Hydroxycinnamic acid Flavonoids

Plants produce phenolic compounds, which are further broken down into phenolic acids and polyphenols, as a major class of secondary metabolites. These substances can arise as derivatives, such as ester or methyl esters, or they can be found in combination with mono- and polysaccharides, connected to one or more phenolic groups. The phenolic acids, flavonoids, and tannins are thought to be the primary dietary phenolic components among the several types of phenolic compounds. Numerous studies have found a strong and positive association (p 0.05) between the antioxidant capability of fruits and vegetables and the presence of phenolic compounds. When included in the human diet, this antioxidant mechanism found in plants plays a significant role in lowering the danger of lipid oxidation in tissues (both plant and animal), as it not only preserves the quality of the food but also lowers the likelihood of contracting certain diseases. According to studies, a diet high in fruits and vegetables helps slow down the aging process and lowers the risk of chronic diseases linked to inflammation and oxidative stress.

The ability of phenolic compounds to scavenge free radicals, donate electrons or hydrogen atoms, or bind metal cations is thought to be the basis for their antioxidant action [9]. The ability of phenolic compounds to inactivate free radicals is conferred by their molecular structures, specifically the amount and locations of their hydroxyl groups and the types of substitutions they undergo on their aromatic rings. This relationship between structure and activity is known as the structureactivity relationship (SAR). These compounds' significant antioxidant activity is due to the hydrogen atoms of the neighboring hydroxyl groups (o-diphenol), which are positioned in various places of the rings A, B, and C, the double bonds of the benzene ring, and the double bond of the oxo functional group (-C=O) of certain flavonoids (Figure 1). Quercetin and catechin have this property. The amount of hydroxyl groups in both compounds are identical and are

located in the same locations. However, quercetin also has a 2,3-diamond in its C ring and a 4 oxo function [10]. Comparing this structure to the saturated heterocyclic ring of catechin, which has about half the antioxidant activity, the advantage is an increase in the TEAC (Trolox equivalent antioxidant capacity) value.

In addition to quantity, other factors that affect the functioning and stability of phytochemical compounds in the human body include their placement in the food matrix, their proximity to other bioactive substances, and their interactions and bonds with other molecules. The release of these compounds during food preparation, such as through the application of high temperatures or freezing, is inferred by a rise in their bioavailability in the human body. Phenolics are present in plants either bound to the cell membranes or walls or free. According to some investigations, heating alters the extractability of various polyphenols, particularly flavonoids, which changes how much of them are present. In this manner, cooking would make it easier for polyphenols attached to the wall to be released than they would be from the raw material. Other writers concur that the heat-induced release of these chemicals, as observed in citrus peel and sweet corn, occurs as a result of matrix disintegration.

1.3.3.1 Curcumin

The turmeric plant, a flowering member of the ginger family best known as a curry spice, contains curcumin, a yellow pigment that has anti-inflammatory qualities and the capacity to boost the body's production of antioxidants. Curcumin and the curcuminoids found in turmeric can be extracted to produce supplements that have a much higher potency than turmeric. However, curcumin is absorbed poorly during digestion, so a myriad of different formulations have been created to improve its bioavailability.

The body's endogenous antioxidant levels rise when curcumin is supplemented, and inflammation-related biomarkers are decreased. Even though curcumin needs more study in many health-related areas, the evidence that is currently available points to modest to slight improvements in osteoarthritis pain and function as well as in the signs and symptoms of depression and anxiety. It is feasible to lower LDL cholesterol, blood glucose, and blood pressure, although the evidence on these effects is sparse and inconsistent.

The fact that curcumin absorbs poorly when taken orally on its own is one of its biggest disadvantages. Up to 8 grams of curcuminoids have not been linked to major negative effects in people in terms of potential negative consequences. Long-term research with more thorough evaluations are nevertheless required. High doses of curcumin have been used in studies, and some modest side effects have been noted, including as nausea, diarrhea, headaches, skin rashes, and yellow stools. Because piperine dramatically increases intestinal permeability, using curcumin alongside it may result in negative pharmacological responses. Different curcumin formulations have undergone varying levels of safety testing.

The potential beneficial effects of curcumin seem to be mainly the result of its anti-inflammatory and antioxidant properties. These properties are mediated by curcumin's direct or indirect interaction with (and modulation of) various molecular targets, including transcription factors, enzymes, cell cycle proteins, receptors, cell surface adhesion molecules, growth factors, and protein kinases.

The spice turmeric has drawn a considerable lot of interest from the scientific, medical, and culinary worlds. Turmeric is derived from the perennial herbaceous plant Curcuma longa, which belongs to the ginger family. Although curcumin Figure 1.4, a component of turmeric, has been used medicinally for thousands of years, the precise mechanism of action and the bioactive components have only recently been determined. Curcumin, also known as diferuloylmethane, is the main natural polyphenol present in the rhizomes of Curcuma longa (turmeric) and other Curcuma species. It is 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. Curcuma longa has been used as medicine for millennia in Asian countries because of its anti-inflammatory, antimutagenic, antibacterial, anticancer, and anti-mutagenic properties [18,22].

Figure 1.4

Chemical structure of 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene- 3,5-dione (curcumin)

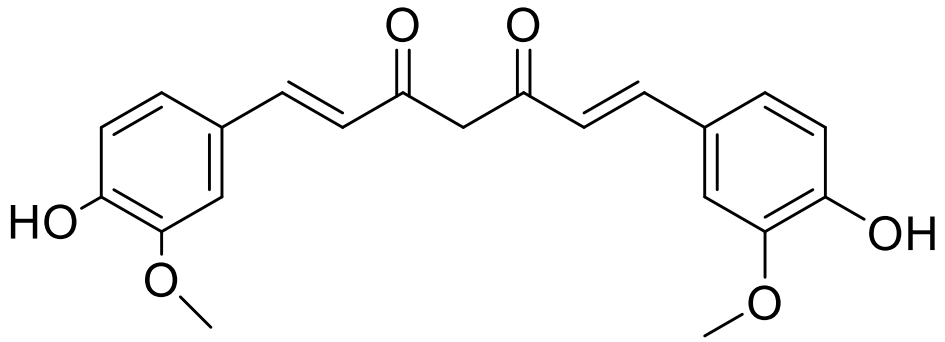
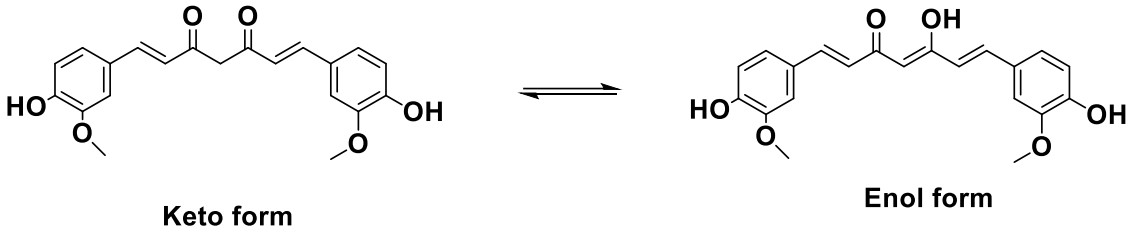


Figure 1.5 shows the structural equilibrium between the enol form and the keto forms of curcumin. This equilibrium is caused by intramolecular H-bonding among the oxygen atom of the keto carbonyl and the hydrogen of the enolic functionality. The solvent's polarity and pH level control the balance between the keto and enol forms. Because of the intramolecular H-bonding and conjugation boost the stability of the enol form, it predominates in water solution under basic circumstances and in organic polar medium (such as ethanol and dimethyl sulfoxide (DMSO).

However, in solid state or nonpolar solvent, and in an acidic or in neutral value of pH, the keto form predominates [23, 27].

Figure 1.5

Keto-enol tautomerism



Indicating selective solubility, the crystalline curcumin powder is relatively hydrophobic and easily soluble in organic medium like ethanol, methanol or in acetone, and DMSO. However, it only dissolves 0.6 g/mL in water. Its therapeutic use and clinical usefulness are constrained due to its very low solubility under physiological environment, high chemical reactivity in addition to low gut absorption, quick metabolism, quick plasma clearance, and significant intestinal degradation. Consequently, methods for chemically

altering curcumin structure have been developed, and their biological activity by creating new curcumin and synthesize it derivatives and analogues. Such structural alterations to the original structure will increase the efficacy as well as the medicinal and physicochemical qualities [28, 35].

1.3.3.1.1 Extraction Of Curcumin

Despite the fact that it has been two centuries since the first attempts to extract and separate curcumin from turmeric powder, highly refined and cutting-edge extraction techniques are constantly being published. The techniques used to extract curcumin can be divided into traditional techniques (like solvent maceration and Soxhlet extraction) and contemporary techniques (like microwave- and ultrasound-assisted extraction), the choice of the best extraction technique is crucial since it is the first step in the qualitative and quantitative examination of the contents of medicinal plants. Even when using the effective chromatographic detection method, an incomplete process of extraction may result in a low yield and inadequate validation [36].

1.3.3.1.2 Extraction Techniques of Curcumin

There are numerous examples of traditional techniques used to extract curcumin from unprocessed plants, including:

1. **Soxhlet extraction method**

This technique extraction and filtration. Both extraction and filtration of the product can be done in one step, making it the most traditional method for extracting curcumin. Curcumin from natural herbs might be extracted using a variety of solvents with different polarity, including acetone, $CHCl_3$, EtOAc, and MeOH [37].

2. **Solvent maceration method**

It is yet another conventional technique for obtaining curcumin. The turmeric oleoresin was produced by macerating the crude plant rhizomes with an organic liquid, followed by the solvent's evaporation. Curcumin is frequently extracted using hexane, EtOAc, acetone, MeOH, and EtOH which is being preferred over the other organic solvents.

The aforementioned standard extraction procedures have several significant drawbacks, including high temperature, prolonged extraction times, low yields, and solvent escape

into the environment. These limitations prevent the use of standard approaches and encourage researchers to create new, cutting-edge extraction methods [38].

3. **Recent extraction methods**

Curcumin was obtained from its natural source by several recent techniques like:

A. **Extraction Using Microwave (ME)**

The target compounds can be extracted from their natural sources faster and more efficiently using the relatively new MAE technology. The foundation of MAE theory is the simultaneous migration of target bioactive compounds through the solvent and out of the matrix in the sample matrix [39]

Because MAE uses little organic solvent, it has been dubbed a green chemical method. High extraction yield, compact equipment, shorter processing times, quicker compound heating, and the use of both polar and non-polar solvents are further benefits of MAE [40].

B. **Ultrasound-assisted extraction (UAE)**

It is a useful method for removing bioactive substances from their undeveloped plants. The pores on the cell wall swell and enlarge as a result of ultrasonic waves, making it easier for the extracting solvent to enter the cell. The resulting microbubbles then expand, collapse, and produce shock waves, which ultimately break cell walls. By doing this, the elements from inside the cells can more easily enter the solvent around them [41].

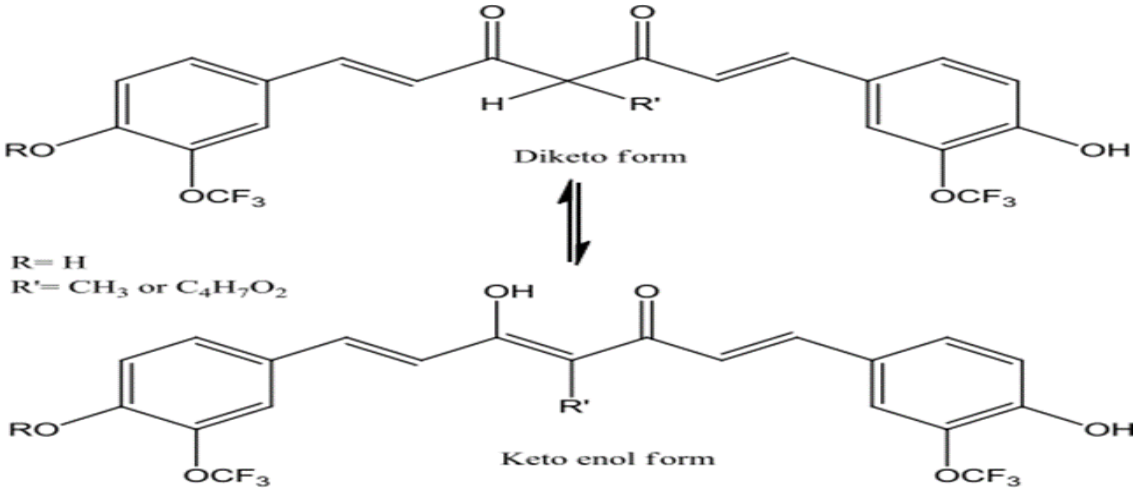
1.3.3.1.3 Curcumin Structural Characteristics

Three chemical functionalities make up the curcumin structure (Figure 1.6), including two phenyl ring systems with o-methoxy and phenolic groups, seven aliphatic carbon linkers, and a carbon chain with an unsaturated diketone moiety [42,43], as shown in Figure 1.5. The IUPAC nomenclature (1E, 6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1 , 6-heptadiene-3,5-dione, curcumin is a symmetrical molecule. It has the molecular weight of 368.38 g/mole and the chemical formula $C_{21}H_{20}O_6$ [44,45]. The biological functions of curcumin are significantly influenced by curcumin tautomerism. For instance, the development of extracellular plaques made of amyloid beta (A) aggregates is the primary pathogenic aspect of Alzheimer's disease. By attaching to these aggregates,

curcumin and its analogues work to prevent the development of Alzheimer's disease. Yanagisawa et al. discovered that the binding activity of curcumin structures analogues with a diketo form as shown in Figure 1.6, was significantly lower than that of curcumin structural analogues with a keto-enol form [46].

Figure 1.6

A curcumin analogue keto-enol tautomerism



1.3.3.1.4 Lab Methods Of Curcumin Synthesis

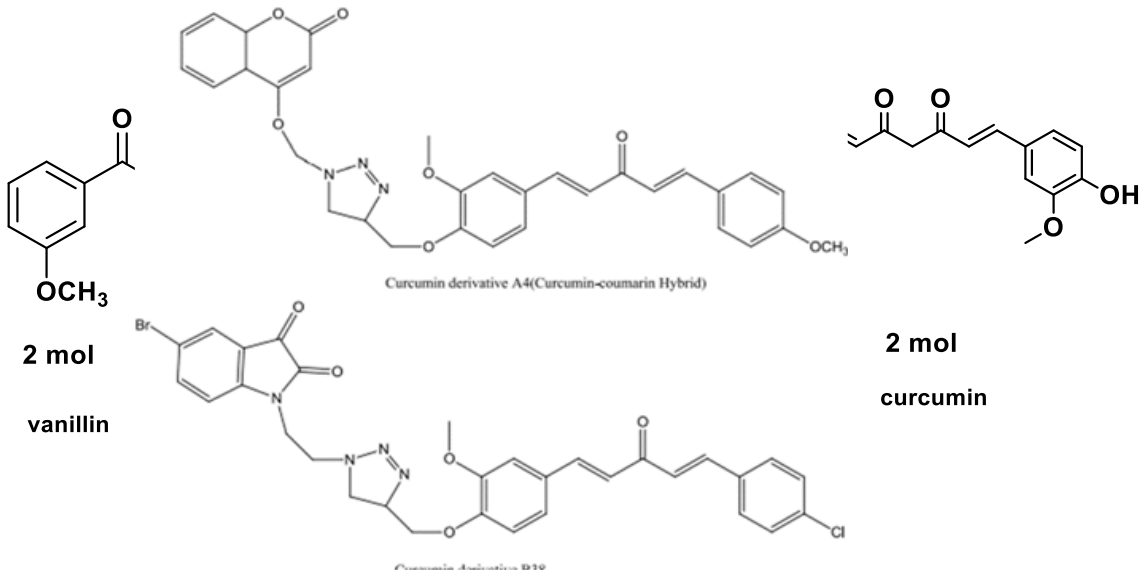
Aldol condensation is a process that can be used to create curcumin as a different method of acquisition. For the creation of new CC bonds, the aldol condensation reaction is a crucial and often employed process in organic synthesis. It plays a significant role in the preparation of unsaturated carbonyl containing compounds, which are widely used in the industrial manufacturing of medicinal drugs [47].

Aldol condensation is possible in either an acidic or basic environment. Typically, a homogeneous catalyst supported on silica in basic medium, such as NaOH and magnesium oxide, is added to carry out the reaction. The purpose of adding homogeneous catalyst is to decrease the temperature of the reaction and speeds up the reaction process [48]

Acetyl acetone and functionalized phenyl aldehydes, such as compound vanillin, can be used as building unit in an aldol condensation to create curcumin, as indicated in Scheme [49].

Figure 1.7

Curcumin synthesis by aldol method

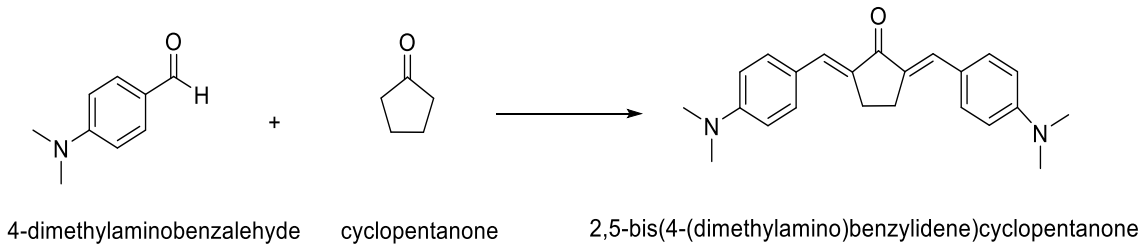


The process of sonication, commonly referred to as ultrasound irradiation, is crucial in the domains of synthetic methods in organic chemistry and the pharmaceutical sector. For many years, irradiation with ultrasonic has gained popularity as a technique for speeding up a variety of chemical reactions through a variety of processes, including improving solubility, accelerating the rate of the reaction, and rejuvenating the surface of reactants and or catalyst.

Through the use of ultrasonic irradiation, Inayaha and his colleagues have created many curcumin mimics. The preparation of the curcumin structure analogue is shown in Figure 1.8 and named as (2E, 5E)-2,5-bis(4-(dimethylamino)benzylidene) cyclopentanone, this analogue showed superior bioactivities compared to other analogues in terms of purity and yield despite the fact that all the structural analogues were produced with a high purity, elevated yield of product, and short period reaction [50].

Figure 1.8

Synthesis of curcumin structures analogue by ultrasonic method



1.3.3.1.5 Bioactivity Of Curcumin

The biological properties of curcumin substance are extraordinary, one of these properties the ability to reduce inflammation and act as an antioxidant, prevent cancer, and fight viruses and bacteria. Curcumin also has a wide range of therapeutic properties, including anti-inflammatory, anti-tumor, anti-apoptotic, anti-angiogenic, cytotoxic, immunomodulatory, antithrombotic, wound-healing, anti-diabetic, anti-stressor, and antilithogenic effects, which contributes to the high demand for this substance [51].

The primary explanation for curcumin medicinal performances is the ability to abstract nitrogen and oxygen free radicals. The methoxy group on curcumin phenyl rings are what provide it enhanced abilities to scavenge free radicals and act as antioxidants when compared to the other two curcuminoids available with curcumin. Because of the H-bonding that takes place among the phenolic hydroxy and O-Me groups of curcumin, it is a superior free radical scavenger. Removing the O-H bond energy and H elimination by free radicals are greatly impacted by this H-bonding. [52]

The antioxidant or prooxidant ability of curcumin in the presence of some metals like Fe(II), Cu(II) or Pb(II) is related to its chelating ability [53], Although curcumin can chelate transition metals through either the O-methoxy phenol or the diketone moiety, chelation is frequently only seen through the diketone functionalities.

It is still unclear what causes and how curcumin actually works to fight tumors. Tetrahydrogenated curcumin demonstrated dramatically improved antioxidant activity but decreased anticancer and antiinflammatory properties after the heptadiene portion of curcumin was hydrogenated [54]

But curcumin cannot be used in clinical settings effectively due to its low bioavailability which caused by low serum level, quick metabolism, restricted tissue distribution, and low solubility [55]

Numerous studies have employed various methods to get over these drawbacks and increase the bioavailability of curcumin, including adjuvants like piperine [56], delivery systems by nanoparticles [57], liposomes [58], and micelles [59].

By altering the curcumin skeleton structure, the compound's biological activity can also be improved. For instance, under simple conditions, curcumin degradation and protons removal can be avoided by using other groups in place of phenolic hydroxyl groups [60].

Several compounds with modified curcumin structures were created over the course of the last few decades, and it was discovered that these provided improved biological activity [61].

In addition to having a low molecular weight and being non-toxic, curcumin has significant anti-cancer properties that make it a promising natural alternative to potential chemotherapeutic derivatives or equivalents [62].

Many other curcumin analogues have now been created. One often employed structural alteration removes the core conjugated beta diketone from curcumin and replaces it with monocarbonyldienone molecules, which are lethal to certain cancers [63].

The diketo moiety of curcumin can combine with a wide range of metal ions, and these metal complexes frequently exhibit greater stability when compared to the parent easily degradable curcumin. In 1997, studies on the first curcumin compounds produced with transition metals having medical value, such as Pt, Pd, In, and Rh, were released. For a variety of molecular targets, the resultant complexes typically displayed beneficial biological activity relative to the parent ligands [64].

The biological activity of curcumin and its derivative metal complexes related to a variety of elements, including the chosen metal, the curcumin [65,66].

1.3.3.1.6 Antibacterial And Antifungal Activities of Curcumin

The rise in bacterial resistance from both gram-positive and negative bacterial to antibiotics and current antimicrobial medications have prompted a pressing need to evaluate and to find substitute medications, such as those made of plant-based substances that has low human cytotoxicity. Even at a high dosage of 8.00 g/day, curcumin is chemical with no toxicity or adverse effects on human health [67].

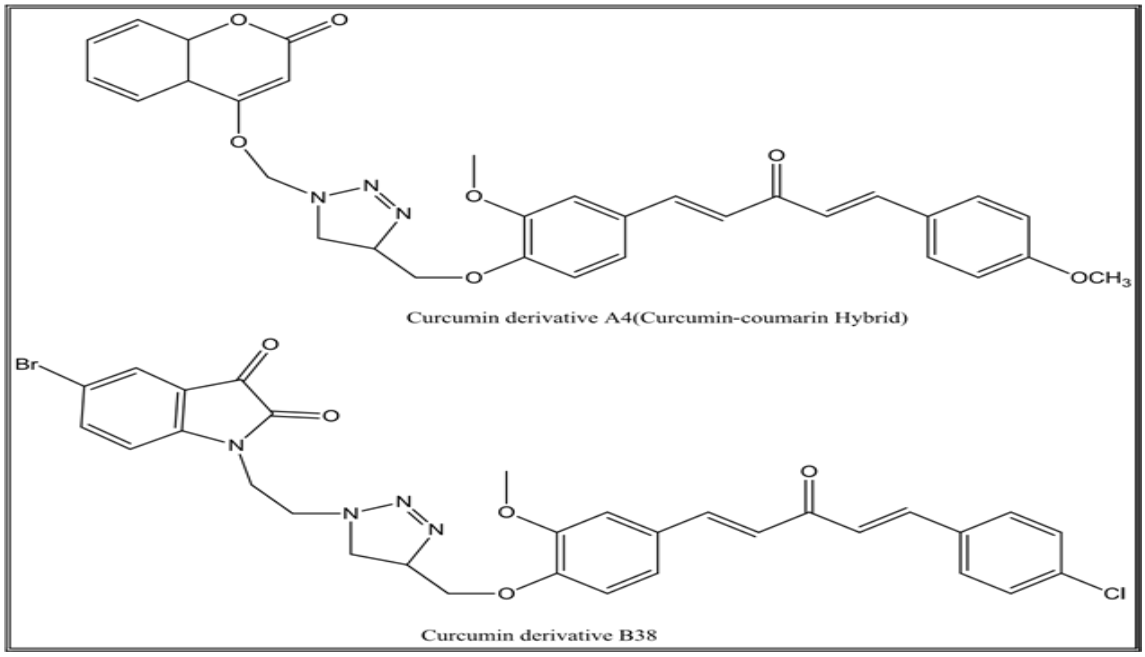
According to recent research [68,69], curcumin may have some antibacterial effects on both methicillin-resistant S. aureus (MRSA) and sensitive S. aureus (MSSA). Curcumin not only has significant synergistic effect as antibacterial action against S. aureus when

administered concurrently with several medications, in addition to its powerful antibacterial efficacy when used alone [70]. Curcumin showed a noteworthy activity against Enterococcus faecalis, E. coli, K. pneumoniae in addition to its effect against S. aureus [71].

A4 and B38, two curcumin derivatives, have demonstrated significant antibacterial activity against S. aureus, Enterococcus faecalis, E. coli, P. aeruginosa in a newly published study led by Singh and his coworkers [72].

Figure 1.9

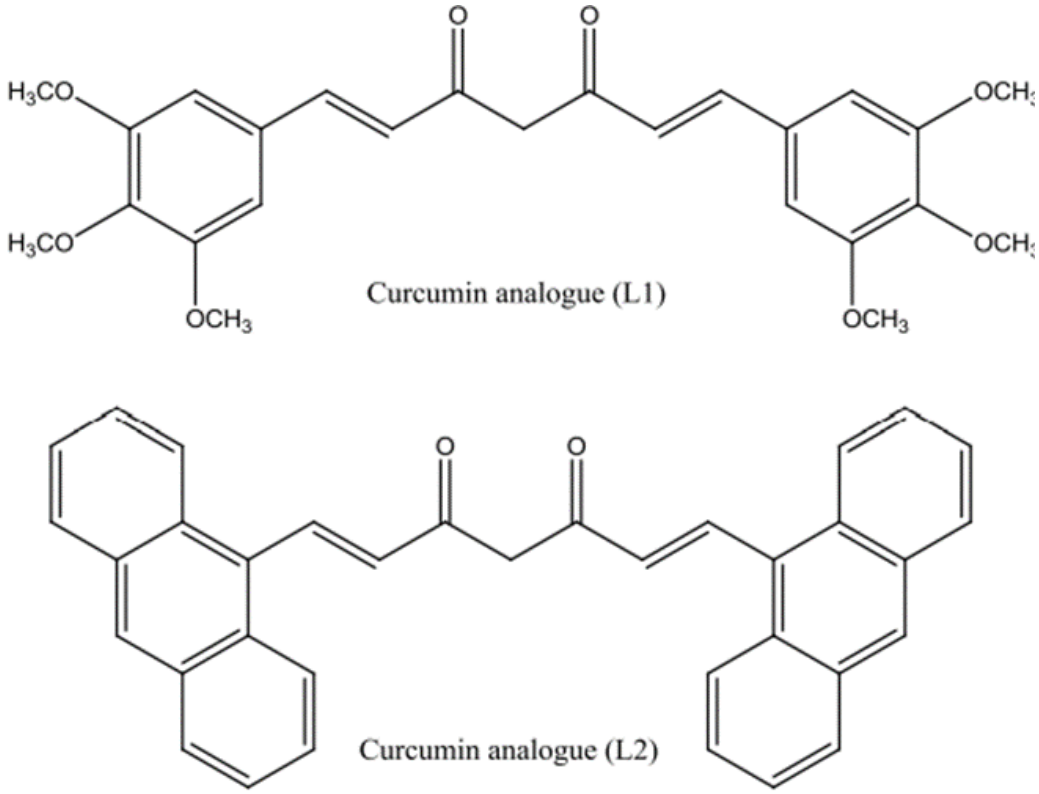
Derivatized with an antibacterial activity



Aspergillus, Penicillium, and Alternaria were the three general against which two curcumin derivatives Fig 1.10, 1,7-Bis (3,4,5-trimethoxy phenyl)- 1,6 -heptadiene-3,5-dione (L1) and 1,7-di (9-anthracenyl)- 1,6- heptadiene-3,5-dione (L2), were investigated for their activity against fungus. The growth of the test cultures has been shown to be potentially inhibited by both analogues, with L1 contributing more favorably than the other manufactured derivative [73].

Figure 1.10

Curcumin analogues with an antifungal activity



1.3.3.1.7 Curcumin And Curcumin Analogues with Antiprotozoal Activity

Numerous parasite forms, including Leishmania [74], Giardia lamblia (75), an Plasmodium falciparum [76], are suppressed by curcumin. Additionally, when administered in conjunction with artemisinin, curcumin exhibits additive antiprotozoal efficacy against Plasmodium falciparum, where they have synergistic antiprotozoal effects [77].

Silvaa and her colleagues created curcumin monocarbonyl derivatives and compared its effectiveness against the trichomoniasis disease caused by Trichomonas vaginalis to that of metronidazole. According to Figure 1.11 -See Appendix (A)-, these curcumin derivatives 3a,3e, and 5e showed high anti-trichomoniasis efficacy on par with metronidazole. Additionally, these compounds have demonstrated greater anti-trichomoniasis effectiveness and improved chemical stability compared to natural curcumin [78].

1.3.3.1.8 Curcumin Analogues with Anticancer Activity

It has been observed that curcumin reduces the benzo[a]pyrene-related mutagenesis effects of tobacco smoke condensate [79,80]. In several cancer types, curcumin may reduce cell growth and trigger apoptosis.

Hujaily et al. developed a number of curcumin analogues, and they tested them for anticancer efficacy. Among these, 5-bis (4-hydroxy-3-methoxybenzylidene) -Nmethyl-4-piperidine and 1,7-bis (4-hydroxy-3- methoxybenzylidene)-N-methyl-4-piperidine have chemical names (4-hydroxy-3-ethoxyphenyl) 1,6 heptadien-3,5 dien. In the studied breast cancer cell lines, these analogs were five times more effective as curcumin at triggering apoptosis [81]. -See Appendix (A)-

Numerous studies have demonstrated that curcumin can alter how NF-kB is activated. This unknown factor can lower apoptosis and promote cellular conversion, metastasis, proliferation, radio resistance, chemoresistance, invasion and related inflammation when it is activated by carcinogens [82,83].

The anticancer activity of a newly synthesized curcumin analogue known as JZ534 Figure 1.13 -See Appendix (A)- on lung cancer cell lines has been studied. Through reducing tumor development, inducing apoptosis, and increasing the effect of apoptosis-related proteins, it has demonstrated strong anti-lung cancer action. Furthermore, compared to curcumin, JZ534 demonstrated greater anticancer efficacy against lung cancer cell lines [84].

By reducing the activity and the suppression of numerous enzymes that promote these processes, such as matrix metalloproteinases MMP-9, MMP-2 and curcumin has been demonstrated to act as an anti-metastatic drug and inhibit cancer cell migration and invasion in vitro [85]. Additionally, curcumin has shown to decrease propagation activity in cervical cancer and against prostatic cancer Figure 1.14 -See Appendix (A)-, which may be more effective than curcumin's other anticancer properties in this regard [86].

Curcumin has been shown in numerous studies to have the ability to inhibit and lessen the growth of colorectal colon since 1995 [87-89].

The two curcumin structural analogues, EF 31 and UBS 109, depicted in Figure 1.15 -See Appendix (A)- may significantly decrease the colorectal cancer cell lines, according to a recent study by Rajitha et al. Additionally, these analogues have better water solubility, potency, and pharmacokinetic characteristics than curcumin [90].

1.3.3.1.9 Antioxidant Properties of Curcumin

Curcumin has been shown by Sankar and his colleagues to be able to subtly upregulate antioxidation ability of enzymes like CAT, SOD, and glutathione reductase [91]. When coupled with other antioxidants, curcumin may potentially display synergistic antioxidant action [92].

Three series of derivatized curcumin have been prpeared by Shang and his colleagues Figure 1.16 -See Appendix (A)-, and their antioxidant performance has been compared to that of the curcumin. They discovered that chemicals that contain an o-dimethoxy- or o-diphenoxyl group may have much stronger antioxidant activity than those that do not. [93].

1.3.3.1.10 Curcumin Derivatives with Anti-Inflammatory Properties

Numerous investigations have demonstrated that curcumin has an anti-inflammatory action that is significantly mediated through the reduction of NF-kB. [94,95]. Several inflammatory cytokines, such as necrosis factor (TNF) tumor, IL-6, IL-8, interleukin-1 (IL-1), and interferon, have been shown to be downregulated by curcumin, according to previous studies [96,97]. As seen in Figure 1.17 -See Appendix (A)-, Paulino and his colleagues have created an equivalent of curcumin known as DM1. Nitric oxide synthase (iNOS) and COX2 were significantly suppressed in the evaluation of its effects on the various inflammatory mediators [98].

The antimicrobial activity is one of the biological processes that has most captured our interest. Since there is an urgent need for the creation of a natural product-based reagent that can combat bacterial resistance to antibiotics [99].

In a prior study [100], thy used curcumin to build a variety of curcumin with a heterocyclic ring, like pyrazole, diazepine and isoxazole. Diazepine had the highest antibacterial activity among the created compounds.

Here, we report a straightforward and practical synthetic procedure for producing Schiff bases from curcumin and amino acid via condensation reaction. This work is a continuation of our research into the production of physiologically active curcumin-based amino acids. Gram positive and gram negative bacteria are used to test the synthetic curcumin-based Schiff bases' antibacterial properties.

1.4 Bacteria: Gram Positive and Gram-Negative

The gram stain test and the makeup of the cell wall help distinguish between the two types of bacteria, gram-positive and negative. Hans Christian created the gram staining test in the1884s. Bacteria either stay purple or turn red or pink when the dye and bacteria are in contact. As seen in Figure 1.18 -See Appendix (A)-, the bacteria are gram-positive if they remain purple and gram-negative if they turn pink or red. The following are key differences between gram-positive and gram-negative bacteria, as given in table 1.1.

Table 1.1

Some comparative characteristics of Gram-Positive and Gram-Negative bacteria

Characteristic	Gram-positive bacteria	Gram-Negative bacteria
Cell Wall	A single-layered, smooth cell wall	A double-layered, wavy cell-wall
Cell Wall thickness	The thickness of the cell wall is 20 to 80 nm	The thickness of the cell wall is 8 to 10 nm
Toxin Produced	Exotoxins	Endotoxins or Exotoxins
Resistance to Antibiotic	More susceptible	More resistant
Outer lipid membrane	No outer lipid membrane	Outer lipid membrane present

Being able to differentiate bacterial species is important for a host of reasons, from diagnosing infection or checking food safety, to identifying which species it is that gives a cheese it’s fantastic character. Bacterial species, and even specific strains can be differentiated using a number of molecular techniques such as PCR, quantitative PCR, genome sequencing and mass spectrometry. But even without getting into the molecular nitty gritty, there are phenotypic differences between groups of bacteria that can be used

to differentiate them. This includes characteristics like their shape (bacilli vs cocci for example), growth in particular nutrients and preference for high or low oxygen environments. Depending on the characteristic being studied, bacterial species may be broken down into broad groups, but taken together this information can narrow the possible identities greatly. One such useful classification – if a bacterium is Gram positive or Gram negative- is based on the structure of bacterial cell walls.

1.4.1 Klebsiella Pneumoniae

A gram-negative bacteria called Klebsiella has been linked to a number of medical conditions, including meningitis, pneumonia, bloodstream infections, wound infections, and infections at surgical or wound sites (see figure 1.19 - Appendix (A)-). The antibiotic family known as carbapenems has lately seen an increase in antibiotic resistance in Klebsiella bacteria. Klebsiella bacteria are typically present in human intestines (where they do not cause disease). They can also be found in human excrement. In healthcare settings, Klebsiella infections are frequently acquired by unwell patients receiving treatment for various ailments. The patients most at risk for Klebsiella infections are those whose care necessitates the use of equipment like ventilators (breathing machines) or intravenous (vein) catheters, as well as those undergoing prolonged courses of certain antibiotics. Klebsiella infections typically do not occur in healthy individuals. Patients should wash their hands frequently to prevent the spread of infections, especially before preparing or eating food, touching their eyes, nose, or mouth, changing bandages or dressings on wounds, using the restroom, blowing their nose, coughing, or sneezing, or touching hospital equipment like bed rails, bedside tables, doorknobs, remote controls, or the phone. Some Klebsiella bacteria have developed a significant level of antibiotic resistance. The class of antibiotics known as carbapenems will not effectively treat an illness or kill bacteria when KPC-producing organisms, including Klebsiella pneumoniae, develop the enzyme known as a carbapenemase. Enterobacterales, a common group of bacteria found in the human gut, includes Klebsiella species and can develop carbapenem resistance. CRE, or carbapenem-resistant enterobacterales, is an order of bacteria that is challenging to treat due to their high levels of antibiotic resistance. Unfortunately, when it comes to treating Gram-negative infections that are resistant to conventional antibiotics, carbapenem medicines are sometimes the last resort [103,104].

1.4.2 Escherichia Coli

Escherichia coli is most frequently found in the intestines of humans and animals, as shown in Figure 1.20 -See Appendix (A)-. The great majority of E. coli are actually completely safe and beneficial for a normal human intestinal system. However, some strains of E. coli are pathogenic, which means they can make people sick by either making them throw up or making them sick outside of the digestive system. Certain E. coli strains that can cause diarrhea can also spread when contaminated food, drink, or contact with people or animals occurs. Since E. coli frequently lives in the intestines of warm-blooded animals, it frequently comes into contact with antibiotics, putting it under strong selection pressure that causes it to develop resistance to the antibiotics that its host consumes. This gave rise to the theory that host origin may be determined using the antibiotic resistance patterns of E. coli strains from various hosts. Specific E. coli phylogenetic groups were found to exhibit different levels of antibiotic resistance, regardless of the acquisition of resistance, proving that the genetic background of E. coli also influences its antibiotic resistance pattern, even though this method was later shown to be useless for its intended use.

Water, soils, and wastewater treatment facilities have all been thought of as natural settings that serve as bacterial genetic reactors. It has been suggested that natural settings like water, soil, and wastewater treatment facilities are bacterial genetic reactors where active genetic exchanges between different bacteria frequently take place, much like what happens in the human intestine. Antibiotic resistance genes are typically linked to mobile genetic components, such plasmids and transposons, which can be traded across bacteria from various biological lineages. There have been numerous reports of multi-drug resistant E. coli bacteria in the environment in earlier research, indicating potential dangers to the public's health associated with human activity [105,106].

1.4.3 Staphylococcus Aureus

Staphylococcus aureus, *a gram-positive* bacteria having a spherical shape, where Staphylococcus aureus, a Bacillota member, commonly inhabits in the human being body's microbiota—the skin and the upper respiratory tract. It can grow without oxygen since it is a facultative anaerobe, and it frequently has catalase and nitrate reduction activities. Despite commonly coexisting with humans as a commensal member of the microbiota, Staphylococcus aureus has the potential to turn into an opportunistic

pathogen. It frequently results in food poisoning, sinusitis, and a multitude of skin and respiratory conditions, including abscesses. By creating virulence factors including powerful protein toxins and the creation of a cell-surface protein that binds and inactivates host defense mechanisms, pathogenic strains frequently aid in the spread of disease. Penicillin is the drug of choice for treating S. aureus infections in strains that are susceptible. Penicillin is an antibiotic generated from some Penicillium fungus species. It prevents the development of peptidoglycan cross-linkages, which give bacterial cell walls their rigidity and strength. The enzyme DD-transpeptidase is attached to the four-membered -lactam ring of penicillin. This enzyme, when active, cross-links chains of peptidoglycan that make up bacterial cell walls. When -lactam binds to DD-transpeptidase, the enzyme's activity is inhibited and it is unable to catalyze the synthesis of the cross-links. Cell death results from an imbalance between cell wall production and breakdown. However, penicillin resistance is quite prevalent (>90%) in the majority of the world, and first-line therapy is typically a penicillinase-resistant -lactam antibiotic (such as oxacillin or flucloxacillin). Depending on local resistance patterns, either of which have the same mode of action as penicillin) or vancomycin can be used. Serious infections like endocarditis may be treated with combination therapy with gentamicin, although its usage is debatable due to the significant risk of kidney impairment.The location and severity of the infection will determine how long the treatment will last. RCT evidence has demonstrated that adjunctive rifampicin has not been shown to be superior to standard antibiotic therapy in the management of S aureus bacteremia in the past. When penicillin was initially released in 1943, S. aureus did not frequently exhibit antibiotic resistance. In fact, S. aureus was being grown in a culture in the original Petri dish that Alexander Fleming of Imperial College London used to study the antibiotic properties of the Penicillium fungus. Penicillin resistance was present in 40% of hospital S. aureus isolates by 1950, and it was present in 80% by 1960 [107].

1.4.4 Methicillin-Resistant Staphylococcus Aureus (MRSA)

Methicillin-resistant Staphylococcus aureus, or MRSA gram-positive bacteria, an antibiotic-resistant kind of bacteria figure 1.21 -See Appendix (A)-. Skin infections from MRSA are most common. It can occasionally result in infections such as pneumonia. Severe MRSA infections, which are the body's excessive response to an infection, can develop if untreated. People who have made a visit to hospitals, such as nursing homes

and dialysis facilities, are more likely to contract MRSA. Antibiotic usage that was frequently unwarranted over many years led to MRSA. For colds, the flu, and other viral infections that don't react to these medications, doctors have been prescribing antibiotics for years. Antibiotics don't always completely eradicate the germs they target, so even when they are used correctly, they contribute to the spread of drug-resistant microorganisms. Since bacteria evolve quickly, pathogens that survive treatment with one antibiotic quickly pick up the ability to resist others.

Steps to reduce your risk of MRSA infection: Maintain good hand and body hygiene,clean hands often, and clean your body regularly, especially after exercise, keep cuts, scrapes, and wounds clean and covered until healed, avoid sharing personal items such as towels and razors, and get care early if you think you might have an infection [108,109].

1.4.5 Staphylococcus Epidermidis

Gram-positive cocci that are arranged in clusters that resemble grapes make up the unusually hardy microbe S. epidermidis. It is a catalase-positive, coagulase-negative, facultative anaerobe that can grow through aerobic respiration or through fermentation, generating after an overnight incubation white, raised, cohesive colonies that are about 1-2 mm in diameter. This bacterium also has a weakly positive response to the nitrate reductase test, according to biochemical assays. It can use glucose, sucrose, and lactose to create acid products, is oxidase negative, and is positive for urease synthesis. It will also create gas if lactose is present. Because it lacks the gelatinase enzyme. The kind and intensity of the illness dictate how Staphylococcus epidermidis infection is treated. Parenteral treatment is necessary for patients with systemic infections. More than 80% of the coagulase-negative staph isolates have methicillin resistance IV vancomycin would be the recommended empiric treatment for staphylococcus epidermidis infection since methicillin resistance should be expected. Treatment options can be limited to beta-lactam antibiotics such as nafcillin and oxacillin if the bacterium is methicillin-susceptible. The therapy's length is determined by the clinical presentation. To stop the infection's source, prosthetic and medical devices typically need to be removed [110].

1.5 Antibiotic

In general, the term "antibiotic" refers to any drug used to combat microorganisms derived from the Greek word "anti," "against," "bios," and "life").

In contrast, non-antibiotic antibacterials (such sulfonamides and antiseptics) are wholly prepared. In medical terms, antibiotics are those created naturally (by one microbe battling another). Both types, however, are present in antimicrobial chemotherapy and have the same goals of eradicating or stopping the multiplications of the microorganisms. The phrase "antibacterials" encompasses soaps, disinfectants and antiseptic drugs, in contrast to antibiotics, which are a large class of antibacterial used higly in medicine and rarely in animal food.

A particular class of antimicrobial agent that fights bacteria is an antibiotic. Antibiotic medications are often employed in the treatment and prevention of bacterial infections because they are the most efficient type of antibacterial agent for doing so. The bacteria might be eliminated or prevented from proliferating. Only a few antibiotics also have antiprotozoal properties. Antiviral medications or antivirals are used to treat viruses rather than bacteria because antibiotics are ineffective against viruses like the flu or the common cold.

Multi-drugs combination is a promising and widely used approaches to treat variety of serious diseases including; infectious diseases, cancer, 2 diabetes, and inflammation. Combinations of multi-drugs can also be used in antibiotics to combat highly resistance bacteria to the individual use of normal antibiotics. The efficacy of the combination may be enhanced and more significant than the sum of each drug’s effect individually, which is called synergism, conversely, when the efficacy of the combination reduces the effect of each drug individually this is called antagonism. Using various drugs with several mechanisms and modes of actions may interacts against the target in many unexpected ways and more effectively. There are several possibilities for synergism such as; (1) Enhancing the efficacy and the specificity of the drug, (2) reducing the dosage while maintaining or increasing the efficacy to avoid toxicity, (3) slowing down the drug resistance development, (4) providing selective synergism against target by doing both toxicity antagonism, and efficacy synergism [111-113].

1.6 Drug Combinations That Are Antagonistic and Synergistic

Infectious disorders, cancer, type 2 diabetes, and inflammation are just a few of the significant conditions that can be treated with the help of multiple medications. To tackle germs that have developed a high level of resistance to standard antibiotics, combinations of many medicines can also be utilized in antibiotics. Synergism describes a situation in which the combined efficacy of the drugs is greater and more significant than the total of the effects of each medicine alone, whereas antagonism describes a situation in which the combined efficacy of the drugs has the opposite effect. Using a variety of medications with different mechanisms and modes of action can result in several unexpected and more powerful interactions with the target. Synergism can take many different forms, including (1) Enhancing the efficacy and the specificity of the drug, (2) reducing the dosage while maintaining or increasing the efficacy to avoid toxicity, (3) slowing down the drug resistance development, (4) providing selective synergism against target by doing both toxicity antagonism, and efficacy synergism [114].

1.7 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a powerful, and rapid method for in vitro amplifying specific DNA sequences, to generate more than 100 billion similar copies. Repetitive cycles including denaturation of the template, annealing of the primer (oligonucleotide) and annealed primers extension using DNA polymerase, to produce unlimited copies of DNA segment from a single copy of initial DNA in this method the 16 synthesized products of primer extension in one cycle can serve as a template in the next one. Thus, the number of target DNA copies duplicates at every cycle. Since its discovery PCR has had such versatile applications in both diagnostic and basic aspects of molecular biology [115].

1.8 Electrophoresis

When an electric field is applied, it causes charged molecules or particles in aqueous solutions to move around. The molecules' charges and the strength of the applied electric field both affect how quickly they migrate. As a result, as charged particles and molecules migrate, they each establish a distinct zone. In an anti-convective medium, such as a gel matrix or a viscous fluid, electrophoresis is carried out. As a result, the size of the charged

particles and molecules affects the rate of migration. This method allows for the high resolution fractionation of a material mixture [116].

1.9 Agarose gel electrophoresis

Agarose gel electrophoresis is a simple, and one of the most known electrophoresis techniques that possesses great resolving power. The agarose gel contains microscopic pores that work like a molecular sieve used to separate molecules based upon the size, charge, and shape. Agarose gel electrophoresis is an efficient separation technique that is mostly used in analyzing DNA fragments of diverge sizes ranging from 100 bp to 25 kb that is generated by restriction enzymes. DNA fragments larger than 25 kb is separated using pulse-field gel electrophoresis, meanwhile, polyacrylamide gel electrophoresis is used to effectively separate DNA fragments smaller than 100 bp or more. Agarose gel electrophoresis is also used to separate other charged biomolecules like proteins and RNA. To separate DNA using this technique, the DNA is loaded into pre-cast wells in the gel then a current is applied. The phosphate backbone of the DNA and RNA molecules is negatively charged; thus, the DNA fragments migrate to the positively charged anode when placed in an electric field. DNA molecules are separated according to their size as they have constant mass/charge ratio. The migration rate of DNA molecules through a gel depend on the following; (1) The size of DNA molecules, (2) DNA conformation, (3) type and concentration of agarose, (4) electrophoresis buffer, (5) voltage applied. The DNA molecules can be then visualized using UV light after separation and staining with a specific dye [117].

1.10 Schiff base

Schiff’s bases are compounds with aldehyde- or ketone have an imine group replaced the carbonyl group. These have a variety of biological actions in addition to being frequently employed for industrial applications. These are the most widely used organic chemicals, which are utilized as pigments, catalysts, and dyes intermediates in organic synthesis, polymer stabilizers

A Schiff base is a substance that has the general formula R2C=NR' and is regarded as a subclass of imines; imines are substances that include a double bond between carbon and nitrogen; depending on their structure, imines can either be secondary aldehydes or secondary ketimines.

Aziomethine is frequently referred to as the imine (which refers to secondary aldimines). These substances bear the name Hugo Schiff, an Italian chemist. There are numerous nomenclature systems for these compounds. In organic chemistry, schiff bases are used synthetically in a variety of ways. By attacking the nitrogen atom, acid anhydrides, acid chlorides, and acyl cyanides begin to acylate Schiff bases, which results in the net addition of the acylation agent to the carbon-nitrogen double bond. This kind of reaction has been helpful in the synthesis of natural products. A variety of enzymatic processes involving the interaction of an enzyme with an amino or a carbonyl group of the substrate appear to involve the use of Schiff bases as a key intermediate.

Schiff bases are usually colored and transparent solids. They are used in the determination of metal amounts and in the identification of carbonyl compounds due to their precise melting points.

The carbon-nitrogen double bond in Schiff bases rotates more easily than the carbon-carbon double bond, which allows stereoisomers to transform into each other. The reason for this: polarization occurs in the azomethine bond due to the fact that nitrogen is more electronegative than carbon.

With a few exceptions, the very modest energy difference between the stereoisomers of Schiff bases prevents their isolation. Stereoisomers are isolated if the nitrogen atom is only covered by an electronegative group because this group makes it more difficult for molecules to rotate around the azomethine link. A decrease in polarization and an improvement in the covalent double bond's nature result from the azomethine group's electronegative group pushing the nitrogen atom's negative charges in the direction of the carbon.

Due to the nitrogen atom's unshared electron pairs and the double bond's ability to donate electrons, all compounds with an azomethine group exhibit basic characteristics. Comparing Schiff bases to the analogous amines, poorer basic characteristics are evident. This is due to the fact that while the nitrogen atom in amines experiences sp3 hybridization, when the imine structure is produced, this hybridization transforms into sp2 hybridization. The basicity will significantly diminish when the s character increases in hybridization.

The C = N system is a weak chromophore that shows absorption in the ultraviolet field. Conjugation with phenyl groups shifts absorption to the visible region. When there is a deactivating substituent in the aromatic ring, such as a halogen, the wavelength of absorption decreases. Generally, aryl alkyl ketimines are absorbed at values between dialkyl and diaryl ketimines [5]. The IR stretch bands of the C = N system are generally observed at 1610–1635 cm−1 and that of C = N+ at 1665–1690 cm−1.

Various characteristics of Schiff bases depend on the substituents connected to the azomethine group. When the nitrogen atom is connected to an electronegative group, the stability of the azomethine molecule rises. The finest illustration of this is the fact that Schiff bases with alkyl or aryl substituents on the nitrogen atom are substantially more unstable to hydrolysis than oximes with hydroxyl groups on the nitrogen atom, as well as phenylhydrazone and semicarbazones with NH groups. Despite being resistant to alkalies, Schiff bases are hydrolyzed into amine and carbonyl compounds in an acidic environment.

The Schiff base formation reaction is reversible. As a result of the reaction, one mole of water is formed and the water in the environment shifts the direction of the reaction to the left. Therefore, the reaction is usually carried out in solvents where water can be removed from the environment by distillation, forming an azeotrope. If the reaction is carried out using amines containing an electronegative atom with unpaired electrons in the nitrogen atom, the reaction is completed and since hydrolysis will not occur, Schiff bases can be isolated with high efficiency.

The structures of Schiff bases are determined by the tautomeric transformations that occur depending on the polarity of the solvent and the hydrogen bonds that occur in the molecule. The preferred conformation in terms of the stability of Schiff bases is the nonplanar This conformation has also been confirmed by quantum mechanics calculations [118].

1.11 Genotoxicity

Genotoxic processes alter the structure, segregation, and informational content of the genetic materials (DNA, RNA) in cells, which has an impact on the integrity of the cell. Genotoxins have both radiation and chemical components. Genotoxins are mutagens that

can alter DNA or chromosomal material, which can result in mutation. All genotoxic compounds are mutagens, however not all mutagenic substances are genotoxic. Genotoxins can be divided into two categories based on how they affect the body: (1) agents that cause cancer ("carcinogens"), and (2) agents that cause mutations ("mutagens"). (3) "Teratogens" that cause congenital defects. Congenital impairments are caused by heritable mutations, which occur from genetic damage to germ cells, whereas cancer is caused by genetic damage to somatic cells in eukaryotic organisms. There are many different types of mutations, such as genetic information duplication, deletion, or insertion [119].

1.11.1 Genotoxicity Testing

Genotoxicity study is a variety of an in vitro and in vivo experiments used to find any drug or product that could harm the genetic material indirectly or directly or through a number of different processes. These tests aid in determining the risks associated with DNA fixation and damage. Genotoxicity assays are essential for determining whether novel chemical entities can be cancerous or poisonous by determining whether they test positive [1120,121].

1.12 The Aim of The Work

The main objective of this work develop more potent new antimicrobial drugs.

A new set of curcumin-based Schiff bases synthesized to satisfy the first objective, the antimicrobial of the target compounds on Gram-positive and Gram-negative bacteria investigated.

The structures and the physical properties of the prepared compound analyzed by spectroscopic means (IR, NMR) melting point, and GC/MS.

Chapter Two
Experimental Part

2.1 Materials and procedures

2.1.1 Materials

Unless otherwise shown, all of the chemicals used in this project, including the solvents and reagents ethanol, acetone, dimethyl sulfoxide (DMSO), curcumin, histidine, lysine, cysteine, and arginine, were purchased from Aldrich Chemical Company.

2.1.1.1 Thin layer chromatography (TLC)

Spot visualization was done using UV light. The measurements of melting points, and FTIR spectra were accomplished in The Chemistry Lab /at An-Najah national university. The reaction was monitored by TLC. The purity of the prepared compounds determined by TLC was carried out using plates precoated with silica gel (GF 254 class 60, Aldrich, Jerusalem). A solution consisting of ethyl acetate: hexane (40:60%) was used as an eluting solvent.

2.1.1.2 Fourier transforms infrared (FT-IR)

Schiff bases' FT-IR spectra were captured using a Bruker-Alpha ATR/FTIR spectrophotometer (Germany). The band intensities are described as strong (s), weak (w), board (br), and medium (br), and the band frequency is expressed as wavenumber (cm^{-1}) (m).

2.1.1.3 Nuclear magnetic resonance (NMR)

Proton NMR (H^1-NMR) spectra was used to determine the chemical structures of the prepared Schiff bases. On the NMR, Bruker GmbH-400 MHz, and (H^1-NMR) were recorded (Germany). These spectra's chemical shifts were quantified in parts per million (ppm) in relation to an internal standard called tetramethylsilane (TMS). For the purpose of identifying spin-spin coupling in H^1-NMR, the names singlet [s], doublet [d], triplet [t], and multiplet [m] were used.

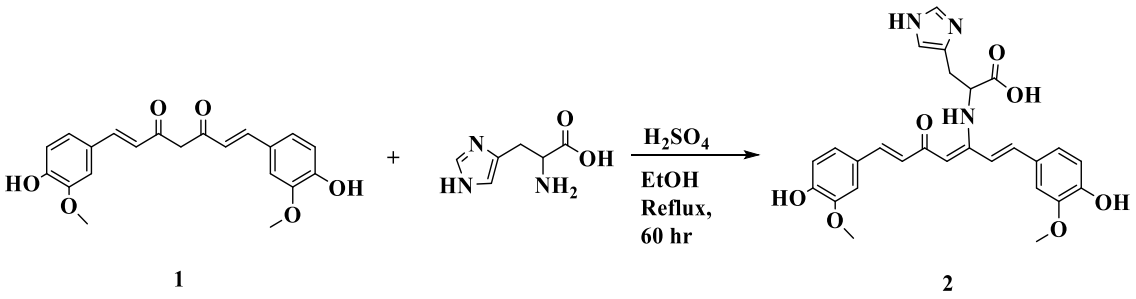
2.1.2 Preparation of Schiff bases

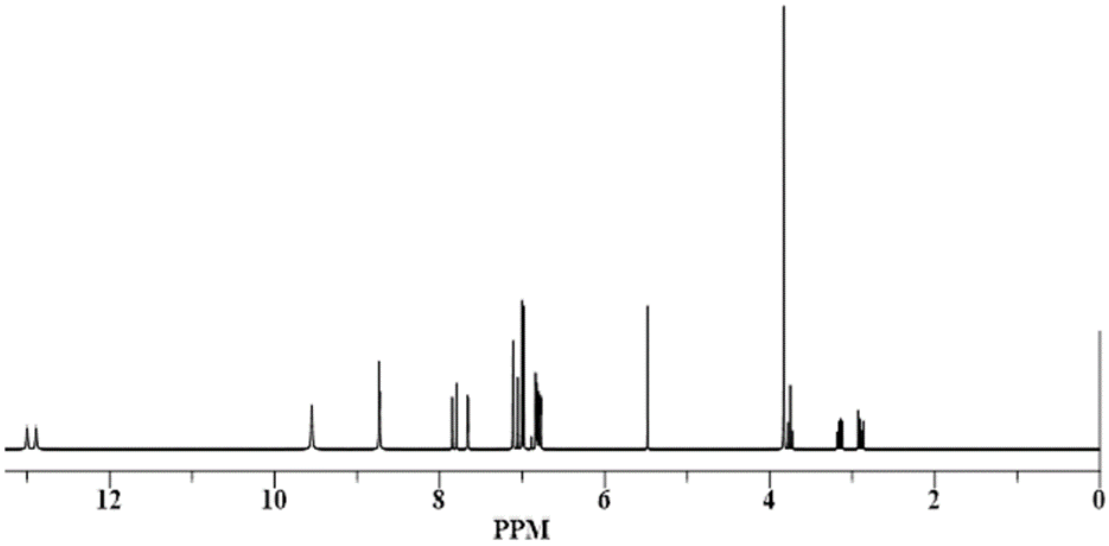
2.1.2.1 Preparation of ((1E,3Z,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,3,6-trien-3-yl)histidine (2)

In a single necked (R.B.F) round bottom flask, a solution of curcumin (1,7- Bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) (5.0 g, 0.0136 mol) in ethyl alcohol (100.0 mL) was made. Histidine compound (2.1 g, 0.0136 mol) and then was added (10.0 drops) of H2SO4. Hexane: Ethyl Acetate (3:2) was used as an eluting solvent while the reaction mixture was continuously stirred under reflux for 60.0 hours. After the reaction was cooled to room temperature the solid was collected by removing the solvent by evaporation under vacuum. The residue was then washed by ($NaHCO_3$, 5% solution) to neutralize residual acid, filtering, and washing it again several times with water before drying. The product was recrystallized with ethanol and water to finish the purification. The product was collected by suction filtration. Yield 82.0%. m.p. 218-221 ^oC, IR (neat): ν_{max} cm^{-1} 1623.2 (C=N), 3374.3 (NH), and 1031.7 (C-O). 1H-NMR (DMSO-d_6) δ: 2.9 (1H, d), 2.91 (1H, d), 3.83 (6H, s, methyl), 5.48 (1H, vinylic), 6.85 (4H, m), 7.03 (3H, m), 7.66 (1H, C5 imidazole), 7.82 (1H, vinylic benzylic), 8.73 (1H, C2, imidazol), 8.73 (1H, d, amino acid, NH); 9.55 (bs, 2H, phenolic, OH), 12.89 (1H, bs, carboxyl), 13.0 (1H, s, NH imidazol).

scheme 2.1

Proton NMR of compound 2





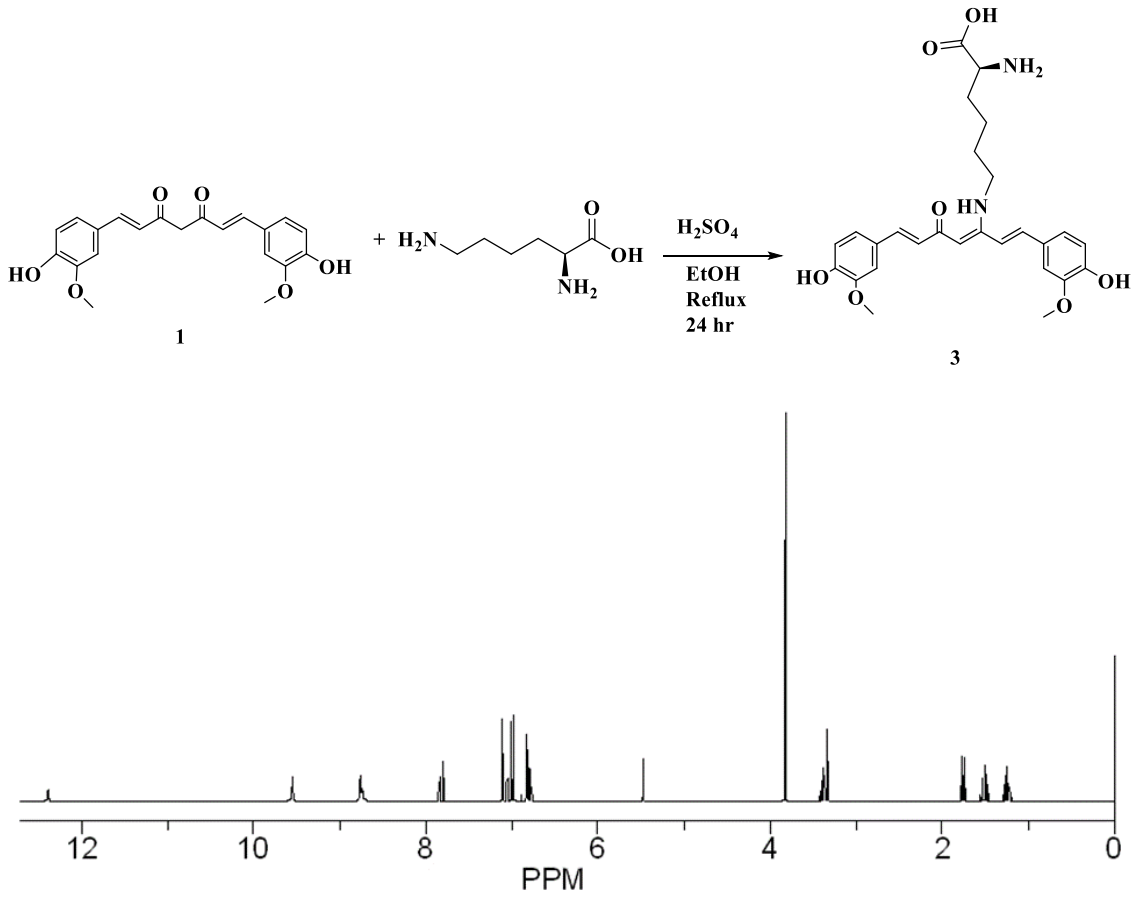
2.1.2.2 Preparation of N6-((1E,3Z,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,3,6-trien-3-yl)-L-lysine (3)

curcumin (1,7- Bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) (5.0 g, 0.0136 mol) was dissolved in ethyl alcohol (100.0 mL) and placed in a round bottom flask with a single neck. It was then added (10.0 drops) of H_2SO_4 and the lysine compound (2.1 g, 0.0136 mol). As an eluting solvent, hexane:ethyl acetate (3:2) was used, and the reaction mixture was stirred under reflux for (24.0 hours) while being continuously observed by TLC. After the reaction was cooled to room temperature the solid was collected by removing the solvent by evaporation under vacuum. The residue was then washed by ($NaHCO_3$, 5% solution) to neutralize residual acid. To complete the purification, the product was recrystallized using ethanol and water. The product was dissolved in a boiling ethanol. The product was collected by suction filtration. Yield 87.3.0%. m.p 213-217 ^oC IR (neat): v_{max} cm^{-1} 1623.2 (C=N), 3374.3 (NH), and 1030.7

(C-O). ^{1}H-NMR (DMSO-d6) δ: 1.25 (t, 2H), 1.5 (m, 2H), 1.76 (m, 2H), 3.4 (1H, m), 3.83 (6H, s, methyl), 5.48 (1H, vinylic), 6.88 (4H, m), 7.03 (5H, m), 7.82 (1H, vinylic benzylic), 8.73 (3H,N-H), 9.55 (bs, 2H, phenolic, OH), 12.4 (H, bs, carboxyl).

scheme 2.2

Proton NMR of compound 3



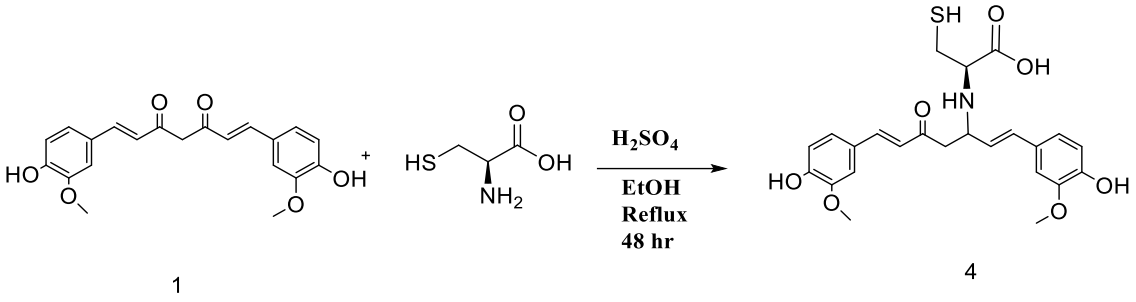
2.1.2.3 Preparation of ((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,6-dien-3-yl)-L-cysteine (4)

In a single necked round bottom flask, a solution of curcumin (1,7- Bis(4-hydroxy-3-methoxyphenyl)hepta -1,6-diene- 3,5-dione) (5.0 g, 0.0136 mol) in ethyl alcohol(100.0 mL) was made. (1.64 g, or 0.0136) mol, of cysteine compound and was then added (10.0 drops of H2SO4. Hexane: Ethyl Acetate (3:2) was used as an eluting solvent, and the reaction mixture was stirred under reflux for (48.0 hours) while being continuously monitored by TLC. After the reaction was cooled to room temperature the solid was collected by removing the solvent by evaporation under vacuum. The residue was then washed by ($NaHCO_3$, 5% solution) to neutralize residual acid, filtering, and washing it

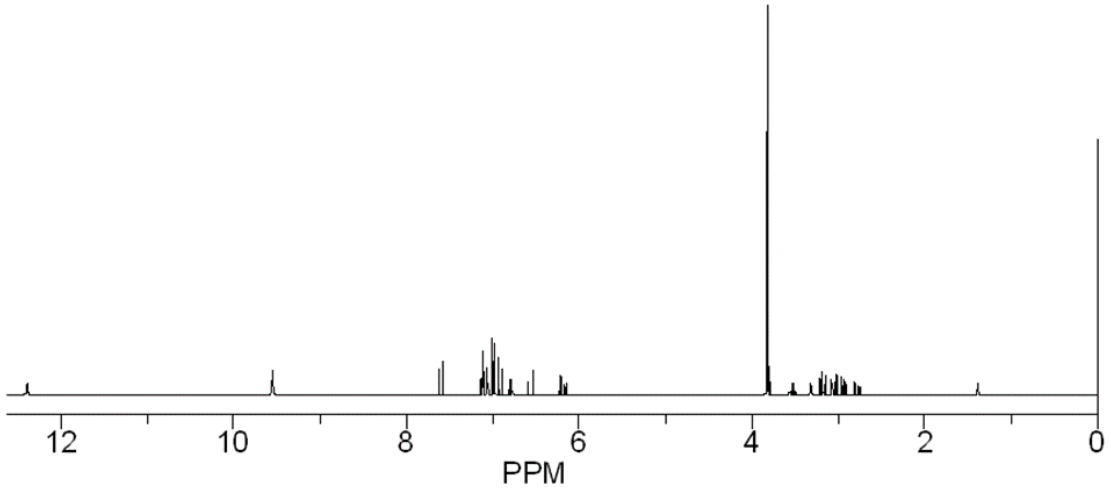
again several times with water before drying. The product was recrystallized with ethanol and water to finish the purification. The product solid was collected by suction filtration. Yield 83.5.3.0%. m.p 198-202 ^{o}C, IR (neat): v_{max} cm^{-1}: 1623.2 (C=N), 3374.3 (NH), and 1030.7 (C-O). 1H-NMR (DMSO-d6) δ: 1.4 (H, bs, S-H), 2.79 (2H, m), 2.94 (2H. m), 3.83 (6H, s, methyl), 6.19 (2H, d), 6.56 (1H, d), 6.8 (1H, d), 6.91 (6H,m), 7.6 (1H, vinylic benzylic), 9.55 (bs, 2H, phenolic, OH), 12.4 (H, bs, carboxyl).

scheme 2.3

Proton NMR of compound 4



1 4



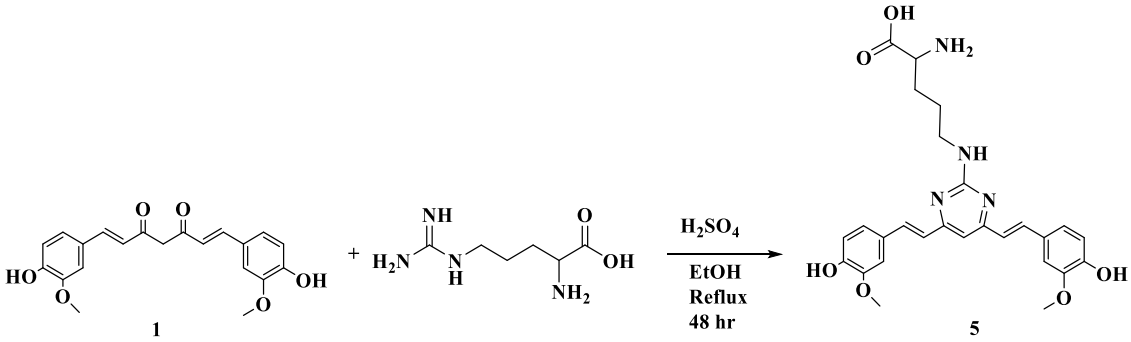
2.1.2.4 Preparation of 2-amino-5-((4,6-bis((E)-4-hydroxy-3-methoxystyryl) pyrimidin-2-yl)amino) pentanoic acid (5)

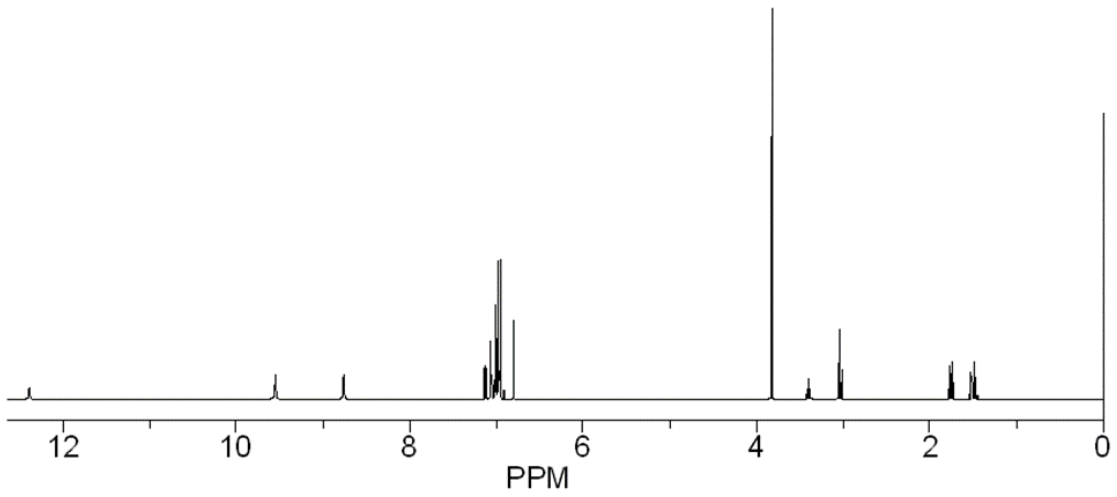
In a single necked round bottom flask, a solution of curcumin (1,7- Bis(4-hydroxy-3-methoxyphenyl)hepta -1,6-diene- 3,5-dione) (5.0 g, 0.0136 mol) in ethyl alcohol (100.0 mL) was made. (2.36 g, or 0.0136) mol, of arginine compound and was then added (10.0 drops of H2SO4. Hexane:Ethyl Acetate (3:2) was used as an eluting solvent, and the reaction mixture was agitated under reflux for (48.0 hours) while being continuously monitored by TLC. After the reaction was cooled to room temperature the solid was

collected by removing the solvent by evaporation under vacuum. The residue was then washed by ($NaHCO_3$, 5% solution) to neutralize residual acid, filtering, and washing it again several times with water before drying. The product was recrystallized with ethanol and water to finish the purification. The product solid was collected by suction filtration. Yield 67.0%. m.p 203-206 ^oC, IR (neat): v_{max} cm^{-1} 1623.2 (C=N), 3374.3 (NH), and 1030.7 (C-O). ^{1}H-NMR (DMSO-d6) δ: 1.5 (2H, m), 1.76 (2H, m), 3.04 (2H. m), 3.4 (1H, t), 3.83 (6H, s, methyl), 6.8 (1H, s, C5 pyrimidine), 6.95-7.0 (4H,m), 7.6 (2H, bs), 9.55 (bs, 2H, phenolic, OH), 12.4 (H, bs, carboxyl).

scheme 2.4

Proton NMR of compound 5





2.1.3 Antibacterial efficacy of the prepared curcumin with Schiff base functionality

2.1.3.1 Media and solutions preparation

The manufacturer's instructions are printed on the bottle of nutritional broth (ACUMEDIA, USA). 2 g of NB and 248 ml of deionized water were combined and

thoroughly dissolved in a 0.5 L bottle. Then the broth divided into 7-ml tubes, each of which was sealed with cotton.

The glass tubes were maintained in the refrigerator at 4.0-6.0 °C after being autoclaved at 121.0 °C for 15.0 minutes.

2.1.3.1.1 Nutrient broth (NB)

The preparation of nutrient broth (ACUMEDIA, USA) was occurred in accordance with the directions listed on the bottle's label by the manufacturer. 2 g of NB and 248 ml of deionized water were combined and thoroughly dissolved in a 0.5 L bottle. The Broth was dispensed into tubes with a capacity of 7 ml each, and sealed with cotton. The glass tubes were placed at 121.0°C for 15.0min, let them cool, and then Kept in refrigerator at 4.0-6.0 °C for use.

2.1.3.1.2 Nutrient Agar (NA)

The manufacturer's instructions were followed while preparing nutrient agar (ACUMEDIA, USA). 500 ml of hot deionized water and 11.5 g of NA were combined in a 1 L bottle until the agar disintegrated. The solution was autoclaved at 121°C for 15 minutes after being allowed to boil for 1 minute. It was then cooled down to about 45.0 °C. Twenty milliliters of agar were placed in each sterile Petri dish, and they were left at room temperature overnight. The Petri dishes were refrigerated at 4°C overnight and were turned upside down the next morning.

2.1.3.1.3 Normal Saline (0.9% NaCl)

In order to make a saline solution with a0.9% NaCl (MW 58.44), 2.25 g of sodium chloride was dissolved in 250.0 ml of distilled water. The saline solution was then divided into tubes with 5–10 ml capacities and their ends were sealed with cotton. The tubes were chilled after being heated at 121.0 °C for 15 min.

2.1.3.1.4 Solution of Dimethyl sulfoxide (10%)

A 10% solution of dimethyl sulfoxide (DMSO) (MW 78.14 g/mol) solution was made in a 0.25 L bottle by combining 10 ml of the chemical with 90 ml of distilled water. After, the solution was autoclaved at 121°C for 15 minutes, and then cooling and being held at room temperature.

2.1.3.1.5 McFarland turbidity standard (No. 0.5)

A 50 µl delete mixture of a 1.17% (w/v) of Barium chloride dihydrate ($BaCl_2$. $2H_2O$) aquoues solution and 9.95 mL of a 1% (v/v) sulfuric acid solution were used to create 0.5 turbidity of the McFarland standard. The tube containing the 0.5 µl solution of McFarland standard was sealed with parafilm to stop evaporation and was kept at room temperature in the dark. The 0.50 McFarland standard was thoroughly mixed using a vortex mixer prior to usage. A 0.5 solution of McFarland Standard is equivalent to a bacterial culture with 1.5×10^8 of colony forming units (CFU)/ml) just like the barium sulfate standards.

To obtain bacterial suspension containing about 1.50×10^8 cfu/mL, four colonies of each bacterium were added into a tube containing 10. mL of sterile normal saline solution 0.9% and mixed well. The turbidity of the suspension containing bacteria was adjusted to be comparable to a 0.5 McFarland standard solution. [122].

2.1.3.2 Used Microorganisms

Five types of bacteria strains have been used in this study. They are summarized in Table 2.1.

Table 2.1

Types of bacterial used in the analysis

Bacterial type of strains	Type of bacteria
S. aureus ATCC 6538P	Gram positive
S. epidermidis ATCC 12228	Gram positive
Methicillin-resistant S. aureus (clinical isolate)	Gram positive
K. pneumoniae ATCC 13883	Gram negative
E. coli ATCC 25922	Gram negative

Two of these bacteria strains are Gram-negative bacteria K. pneumoniae, and E. coli, while the other 3 strains are Gram-positive bacteria included S. aureus, Methicillin-resistant S. aureus (MRSA), and S. epidermidis.

2.1.3.3 Minimum inhibitory concentration (MIC)

The MIC values of curcumin-based Schiff base compounds were evaluated by a broth microdilution process using sterile 96-well microtiter plates as mentioned in the instructions published by Institute for Clinical and Laboratory Standards (CLSI) [123]. The curcumin-based Schiff bases (800.0 $\mu g.ml^{-1}$ of 10.0% Dimethylsulfoxide) and

negative control (10.0% dimethyl sulfoxide) were diluted serially two folds in nutrient broth to a final volume of 100 µL in the wells of the plates. After that, each well received a 1.0 x 10^5 CFU/ml bacterial-inoculum. As negative controls, 100 µL of nutrient broth alone, 100 µL of dimethyl sulfoxide with bacterial inoculum, and curcumin-based Schiff bases, and nutrient broth with no bacteria were used in the tests. Each curcumin-based Schiff base was tested twice. After that, the delete plates were covered and place in an oven at 37 °C overnight. The lowest concentration (highest dilution) of curcumin-based Schiff base compound which caused the bacterial growth to stop was defined as MIC.

2.1.4 The Genotoxic of curcumin-based Schiff bases on E. coli strain ATCC 25922

2.1.4.1 E. coli Inoculation

Some colonies with a 24-hour old strains of E. coli that had been placed in a NA medium were subcultured under sterile envrinmnet in a closed bottle with 25.0 mL of nutrient broth and placed in an oven at 37°C for one hour with mixing. Then, one milliliter of the treated E. coli culture was placed in each of the four sterilized bottles containing 24 milliliters of broth nutrient medium. The bottles were then placed in an oven for additional one hour at 37°C with frequent shaking. The histidine compound was added at different concentrations to each of the four bottles holding the E. coli culture: 0 µg/ml (10% dimethyl sulfoxide), 25 µg/ml, 50 µg/ml, and 100 µg/ml. The bottle which had not histidine compound is considered as a negative or untreated control.

2.1.4.2 Extraction E. Coli DNA

The genome of E. coli was extracted for the enterobacterial repetitive intergenic consensus (ERIC) PCR according to the technique described previously[124]. From each E. coli growth culture four-mL samples were taken after three hours, five hours, and twenty-four hours. The supernatant from each sample was removed after centrifugation at fourteen thousand rpm for 5.0 minutes. The bacterial in pellet form was resuspended in 1 mL of Tris-EDTA containing 10 mM Tris-HCl, and 1 mM EDTA with a pH value of 8, centrifuged for five minutes at fourteen thousand rpm; then, the supernatant was removed. After that, 350 µl of sterile distilled water was re-suspended with the pellet of bacteria, boiled for 15 minutes, then immediately incubated on ice for 5-10 minutes. The samples were centrifuged for 5 minutes at 14,000 rpm and then the supernatant was transferred into new tube.

After that, 350 µl of sterile distilled water was re-suspended with the pellet of bacteria, boiled for 15 minutes, then immediately incubated on ice for 5-10 minutes. The samples were centrifuged for 5 minutes at 14,000 rpm and then the liquid part was moved to a separate tube. After determining the concentration of the DNA in each sample using a nanodrop spectrophotometer, the samples of DNA were kept at - 20°C for ERIC-PCR analysis.

2.1.4.3 PCR analysis of enterobacterial repetitive intergenic consensus (ERIC)

Primer ERIC1: 5'-ATG-TAA-GCT-CCT-GGG-GAT-TCA C-3' and Primer ERIC2: 5-AAG-TAA-GTG-ACT-GGG-GTG-AGC G-3' were used in the ERIC-PCR. Each 25 µ L PCR reaction mixture contained 10.0 mM PCR buffer pH 8.30, 3.0 mM $MgCl_2$, 0.40 mM of each dNTP, 0.80 M of each primer, 1.5 U of Taq DNA polymerase, 5.0% dimethyl sulfoxide, and fixed quantity of DNA (30.0–35.0 µg). Then, using a thermal cycler (Mastercycler personal, Germany) for DNA magnification, which was carried out was carried out under the following thermal conditions: initial denaturation for 3 min at 94°C; followed by 40 cycles of denaturation at 94.0 °C for 50.0 s, annealing at 50°C for 1 min, and extension at 72.0 °C for 1 min; followed by a final extension step at 72.0°C for 5 min. After that, the the amplified PCR fragments were examined examined using 1.5% agarose gel electrophoresis.

Ethidium bromide with a concentration of 0.5 µg/ml of water was used to stain the gel. A UV trans-illuminator was used to observe the ERIC-PCR profile, and it was subsequently photographed by gel documentation system. Alterations in the band pattern of ERIC-PCR following a curcumin-based heterocyclic histidine molecule treatments, including variations in band intensity as well as gain or loss of bands, were taken into consideration [125,126].

Chapter Three
Results And Discussion

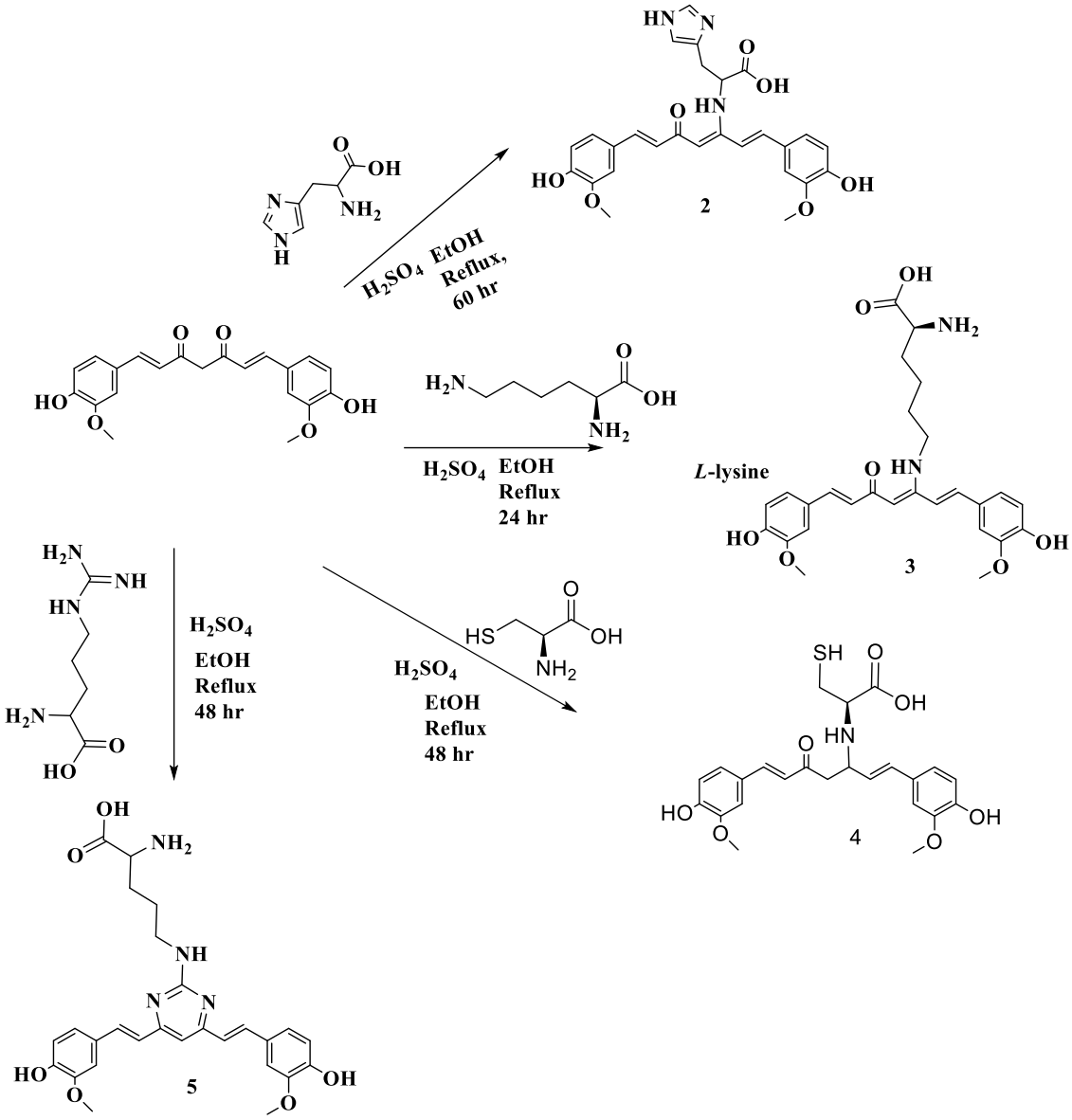
3.1 Curcumin with Schiff base functionality

In a prior study conducted in our labs, several curcumin-based heterocyclic compounds were created [127] by reacting curcumin with different diamino compounds. Curcumin and the diamino compound were combined and heated under reflux for two hours to complete the synthesis. Sulfuric acid served as a catalyst and a solvent at the same time. Some of the curcumin-based good heterocycles that were created had strong antibacterial effects against Gram positive bacteria and weak effects against Gram negative ones. This work involves creating a number of novel curcumin-based Schiff bases by reacting curcumin with various amino acids.

A novel procedure was used to synthesis the target Schiff bases, and in some instances, as demonstrated in the experimental section, there were quantitative yields. Using ethanol and catalytic amounts of sulfuric acid, the amino acids and curcumin were reacted with the loss of water molecule to form the product. A general scheme showing the prepared curcumin-based Schiff bases is shown in scheme 3.1.

scheme 3.1

A summary of the prepared curcumin-based Schiff bases



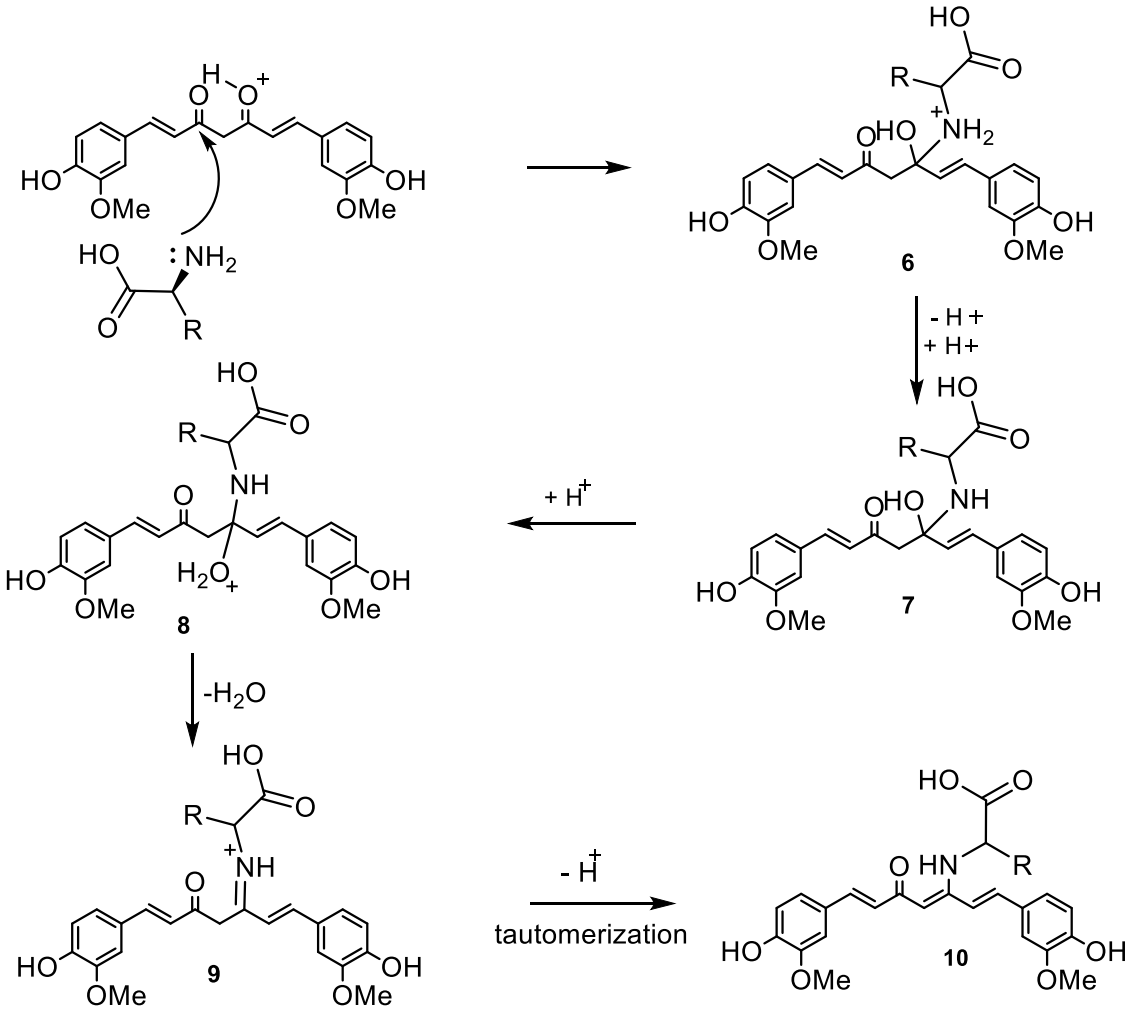
By condensation mechanism of curcumin compound with different amino acids in a (1:1) ratio utilizing the ethyl alcohol as a solvent and H_2SO_4 as a catalyst, curcumin with Schiff base activity was created. TLC was used to keep tabs on the reaction's development. By crystallization, the products were cleaned form impurties as stated in Chapter 2 (experimental) part. FT-IR of the prepared curcumin-based Schiff bases (see figure 3.1-3.4 - Appendix (A)-).

3.2 Curcumin-based Schiff base mechanism of formation

A step-by-step pathway of the condensation mechanism of the reaction between the reactant curcumin (1) and the amino acid compounds to produce Schiff bases is shown in scheme 3.2 -See Appendix (A)-. The amine group of the amino acid attacks the carbonyl groups of the curcumin, then a loss of water molecule occur (condensation reaction) and tautomerization to form the target molecule. The formation of pyrimidine 5 involves double condensation reaction as shown in the scheme below.

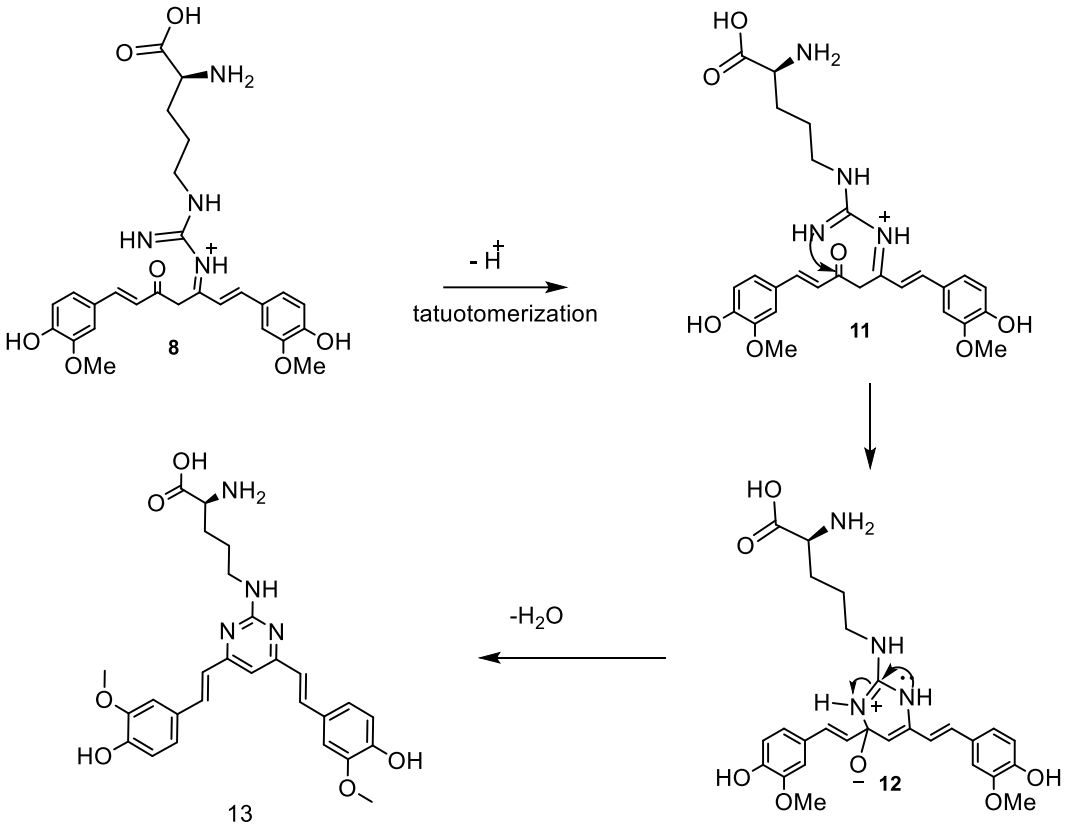
scheme 3.2

A schematic diagram shows the reaction mechanism for the formation of Schiff bases



scheme 3.3

A mechanism showing the formation of pyrimidine 5



3.3 Curcumin Schiff bases as antimicrobial agents

The MIC values of each curcumin Schiff base against five distinct types of bacteria were detected by broth microdilution method. The MIC value of curcumin Schiff base on these different bacterial types had a range 200 µg/ml to 400 µg/ml, except compound 4 had MIC >400 µg/ml. Results showed that compound 2 had the lowest activity (MIC 400 µg/ml) against these types of bacteria. Escherichia coli considered less sensitive to these compounds (MIC 400 µg/ml) in comparison to other types of bacteria. However, S. aureus more sensitive than other types of bacteria, which had MIC 200 $\mu g.ml^{-1}$ against compounds 3, 4 and 5 and 400 $\mu g.ml^{-1}$ against compound 2. The MIC profile of the studied curcumin Schiff base compounds different microorganisms is shown in Table 3.1.

Table 3.1

MIC results of the studied Curcumin-Schiff base against different microorganisms

Schiff base	MIC obtained value (μg.ml^{-1})				
	K. pneumoniae	E. coli	MRSA	S. aureus	S. epidermidis
2	400	400	400	400	400
3	400	400	200	200	400
4	400	400	400	200	200
5	200	400	400	200	200

3.4 The Genotoxicity of Schiff base 5 against the DNA of bacteria E. coli

Figure 3. 5 -See Appendix (A)- shows the obtained profile of the ERIC-PCR product of the DNA isolated from E. coli bacterial in presence and absence of Schiff base 5 over various time periods.

5 for various time periods are shown in the TERIC-PCR profile. A 100-bp DNA ladder is in Lanes L. (negative control). E. coli treated with Schiff base 2 at concentrations of 100 g/ml, 50 g/ml, and 25 g/ml are shown in lanes 1, 2, 3, and 4, respectively. Untreated E. coli strain or control is in lane 4.

Alterations in the extracted genomic DNA from E. coli strain treated with Schiff base compound 2 were evaluated and compared with negative (untreated Schiff base compound 2 controls at the same time intervals.

The number of bands in both treated and untreated E. coli did not change after two hours, according to the results of the ERIC-PCR test. However, compared to the bands from the untreated control, the ERIC-PCR bands generated from the E. coli strain treated with chemical 2 at a dose of 100 μg/mL are more intense. However, at a lower dose of 50 μg/mL, the bands' intensities in the ERIC-PCR product of the Schiff base 2 treated and untreated E. coli strain did not alter.

According to the ERIC-PCR data obtained after 5 hours, the bands made by the E. coli strain treated with Schiff base 2 at concentrations of 100 μg/mL and 50 μg/mL are generally shows more intense bands than the others made by the untreated control. A band with an approximate 250-bp amplicon size was faint in E. coli treated with Schiff base 2 at concentrations of 25 μg/mL and 50 μg/mL in compared to the same band in the

untreated control. Furthermore, treatment with 50 µg/mL resulted in the appearance of a new band with an amplicon length above 500 bp.

After 24 hours, it was discovered that the bands' number and intensities at each concentration (100 µg/mL, 50 µg/mL, and 25 µg/mL) were identical to those of the control bands and had not changed. This might be attributed to bacterial enzymes degrading compounds, or it could be explained by specific mechanisms used by bacteria to be able to repair DNA damage.

The differences in bands intensity and the appearance of specific bands in the tested E. coli compared to the untreated control were the main changes. DNA changes such single and/or double breaks in strand, chromosomal rearrangements and/or point mutations can be caused by genotoxic substances. The sites of primer annealing and/or inter-priming distances may be affected by these modifications or damages to the bacterial DNA [128-131].

Point mutations, significant deletions, and/or homologous recombination are thought to be mechanisms that can create new primer annealing sites and, as a result, produce additional new bands or alter the size of the amplicon [132]. It is challenging to comprehend and pinpoint the precise mechanisms that cause differences in the ERIC-PCR profile, so it is necessary to use additional methods, such as amplicon analysis using DNA sequencing or probing [133], to support and further our understanding of the hypothesized mechanisms. Based on the results of the genotoxicity study, In vivo genotoxicity assessment or with the presence of liver extract is recommended to evaluate the safety of using Schiff base 2 for therapeutic purposes.

3.5 Conclusion

Using a straightforward one-pot procedure, a new series of curcumin-based Schiff bases were created with a quantifiable yield. The curcumin-based Schiff bases were created by reacting curcumin with different amino acids in the presence of sulfuric acid in a catalytic amount. Multiple spectroscopic techniques were used to confirm the structures of the synthesized Schiff bases. The synthesized curcumin-based Schiff bases antibacterial efficacy was assessed against three gram-positive and two gram-negative microorganisms. They showed a range of potency against the examined species, with a

MIC value of 200-400 µg mL-1, Schiff base 5, which was created by reacting curcumin and arginine, had the most effectiveness against the studied microorganisms. The findings of the genotoxic study demonstrated that Schiff base 2, which was created by reacting curcumin and histidine harmed the DNA of E. coli. The potency of compound 2, could be attributed to the multi-N atoms present on the structure.

References

1. Carlson EE, (2010). Natural products as a chemical probe. ACS Chem Biol; 5(7): 639-653.

2. Leroy O, Gangneux JP, Montravers P, Mira JP,Gouin F, Sollet JP, et al., (2009). Epidemiology, management, and risk factors for death of invasive Candida infections in critical care: a multicenter, prospective, observational study in France (2005-2006). Crit Care Med; 37: 1612- 1618.

3. Li J, Larregieu CA and Benet LZ, (2016). Classification of natural products as a source of drugs according to the biopharmaceutics drug disposition classification system (BDDCS). Chin J Nat Med; 14(12): 888-897.

4. Gragg GM and Newman DJ, (2013). Natural products: A continuing source of novel drug leads. Biochimica et Biophysica Acta; 1830(6): 3670-3695.

5. Mustafa YF, Oglah MK and Bashir MK, (2020). Conjugation of sinapic acid analogues with 5- Fluorouracil: Synthesis, preliminary cytotoxicity, and release study. Sys Rev Pharm; 11(3): 482-489.

6. Dar RA, Shahnawaz M, Rasool S and Qazi PH, (2017). Natural product medicines: A literature update. J Phytopharmacol; 6(6): 340- 342.

7. Pott DM, Osorio S and Vallarino JG, (2019). From central to specialized metabolism. An overview of some secondary compounds derived from primary metabolism for their role I conferring nutritional and organoleptic characteristics to Fruit. Front Plant Sci; 10: 835.

8. Kinghorn A, Chin Y and Swanson SM, (2009). Discovery of natural product anticancer agents from biodiverse organism. Curr Opin Drug Discov Devel; 12(2): 189-196

9. Isah T, (2019). Stress and defense responses in plant secondary metabolites production. Biol Res; 52(39).

10. Wangchuk P, (2018). Therapeutic applications of natural products in herbal medicines, biodiscovery programs, and biomedicine. JBAPN; 8(1): 1-20.

11. Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, et al., (2018). Natural products for drug discovery in the 21st century: Innovation for novel drug discovery. Int J Mol Sci; 19(6): 1578.

12. Newman DJ and Cragg GM, (2016). Natural products as sources of new drugs from 1981 to 2014. J Nat Prod; 79: 629-661.

13. Nawaz A, (2011). Curcumin: a natural product of biological importance. Gomal University Journal of Research; 27(1): 7-14.

14. Saleem M.,Nazir M., Ali M.S., Hussain H., Lee Y.S., Riaza N., Jabbar A., Antimicrobial natural products: an update on future antibiotic drug candidates, Natural Product Reports, 27, P 238–254, (2010).

15. Cushnie T., Cushnie B., Lamb A.J., Alkaloids: an overview of their antibacterial, antibiotic-enhancing, and antivirulence activities, International Journal of Antimicrobial Agents, 44(5), P 1-45, (2014).

16. Tungmunnithum D., Thongboonyou A., Pholboon A., Yangsabai A., Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview, Medicines, 5(93), P 1- 16, (2018).

17. Khoddami A., Wilkes M.A., Roberts T.H., Techniques for Analysis of Plant Phenolic Compounds, Molecules, 18, P 2328-2375, (2013).

18. Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: A review of its effects on human health. Foods, 6(10), 92.

19. K. Indira Priyadarsini, Chemical and Structural Features Influencing the Biological Activity of Curcumin, Current Pharmaceutical Design, 2013, 19, 2093-2100.

20. Jianguo Fang, Jun Lu, and Arne Holmgren, Thioredoxin Reductase Is Irreversibly Modified by Curcumin, THE JOURNAL OF BIOLOGICAL CHEMISTRY, 280, 2005, pp. 25284–25290.

21. Rita M. Borik, Nagwa M. Fawzy, Sherifa M. Abu-Bakr and Magdy S. Aly, Design, Synthesis, Anticancer Evaluation and Docking Studies of Novel Heterocyclic Derivatives Obtained via Reactions Involving Curcumin, Molecules, 2018, 23, 1398. doi:10.3390/molecules23061398.

22. Roxana Ciochina, Chasity Savella, Brianna Cote. Davis Chang, and Deepa Rao, Synthesis and Characterization of New Curcumin Derivatives as Potential Chemotherapeutic and Antioxidant Agents, DRUG DEVELOPMENT RESEARCH, 75, (2014) ,88–96.

23. Mahmood Ahmed, Muhammad Abdul Qadir, Muhammad Imtiaz Shafiq, Muhammad Muddassar, Abdul Hameed, Muhammad Nadeem Arshad, Abdullah M. Asiri, Curcumin: Synthesis optimization and in silico interaction with cyclin dependent kinase, Acta Pharm., 67 (2017) 385–395.

24. Jaggi Lal, Sushil K. Gupta, D. Thavaselvam, Dau D. Agarwal, Synthesis and pharmacological activity evaluation of curcumin derivatives, Chinese Chemical Letters, 27 (2016) 1067–1072.

25. Qingyong Li, Jian Chen, Shuyue Luo, Jialin Xu, Qiaoxian Huang, Tianyu Liu, Synthesis and assessment of the antioxidant and antitumour properties of asymmetric curcumin analogues, European Journal of Medicinal Chemistry (2015.), doi: 10.1016/j.ejmech.2015.02.005.

26. Satyendra Mishra, Sejal Patel, and Chandni G Halpani, Recent Updates In Curcumin Pyrazole and Isoxazole Derivatives: Synthesis and Biological Application, Chem. Biodiversity 10.1002/cbdv.201800366.

27. Rama I. Mahran,Magda M. Hagras, Duxin Sun, and Dean E. Brenner, Bringing Curcumin to the Clinic in Cancer Prevention: a Review of Strategies to Enhance Bioavailability and Efficacy, The AAPS Journal, (# 2016) DOI: 10.1208/s12248-016-0003-2.

28. Nikola Hadzi-Petrushev, Jane Bogdanov, Jovanka Krajoska, Jovana Ilievska, Biljana Bogdanova-Popov, Elizabeta Gjorgievsk, Vadim Mitrokhinc, Ramadan Sopie, Hristo Gagov, Andre Kamkin, Mitko Mladenov, Comparative study of the antioxidant properties of monocarbonyl curcumin analogues C66 and B2BrBC in isoproteranol induced cardiac damage, Life Sciences, 197 (2018) 10–18.

29. Luyang Ding, Shuli Ma, Hongxiang Lou, Longru Sun and Mei Ji, Synthesis and Biological Evaluation of Curcumin Derivatives withWater- 136 Soluble Groups as PotentialAntitumor Agents: An in Vitro Investigation Using Tumor Cell Lines, Molecules, 20, 21501–21514, 2015; doi:10.3390/molecules201219772.

30. P.V.Sri Ramya, Lalita Guntuku, Srinivas Angapelly, Chander Singh Digwal, Uppu Jaya Lakshmi, Dilep Kumar Sigalapalli, Bathini Nagendra Babu, V.G.M. Naidu, Ahmed Kamal, Synthesis and biological evaluation of curcumin inspired imidazo[1,2-a]pyridine analogues as tubulin polymerization inhibitors, European Journal of Medicinal Chemistry (2017), doi: 10.1016/j.ejmech.2017.11.010.

31. Mustafa Usta, Heleen M. Wortelboer, Jacques Vervoort, Marelle G. Boersma, Ivonne M. C. M. Rietjens, Peter J. van Bladeren, and Nicole H. P. Cnubben, Human Glutathione S Transferase-Mediated Glutathione Conjugation of Curcumin and Efflux of These Conjugates in Caco-2 Cells, Chem. Res. Toxicol. 2007, 20, 1895–1902.

32. Sujuan Wang, Xixi Peng, Liangliang Cui, Tongtong Li, Bei Yu, Gang Ma, Xinwu Ba, Synthesis of water-soluble curcumin derivatives and their inhibition on lysozyme amyloid fibrillation, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy (2017), doi: 10.1016/j. saa. 2017.09.010.

33. Siti Noor Hajar Zamrus, Muhammad Nadeem Akhtar, Swee Keong Yeap, Ching Kheng Quah, Wan Sin Loh, Noorjahan Banu Alitheen, Seema Zareen, Saiful Nizam Tajuddin, Yazmin Hussin and Syed Adnan Ali Shah, Design, synthesis and cytotoxic effects of curcuminoids on HeLa, K562, MCF 7 and MDA MB 231 cancer cell lines, Chemistry Central Journal, (2018) 12-31.

34. Wenjie Zhang, Heyuan Bai, Liqiang Han, Han Zhang, Bo Xu, Jingrong Cui, Xin Wang, Zemei Ge, Runtao Li, Synthesis and biological evaluation of curcumin derivatives modified with α-amino boronic acid as proteasome inhibitors, Bioorganic & Medicinal Chemistry Letters, 28, (2018) 2459–2464.

35. Rama I. Mahran,Magda M. Hagras, Duxin Sun, and Dean E. Brenner, Bringing Curcumin to the Clinic in Cancer Prevention: a Review of Strategies to Enhance Bioavailability and Efficacy, The AAPS Journal, (# 2016) DOI: 10.1208/s12248-016-0003-2.

36. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines, 8(1).

37. Revathy, S., Elumalai, S., & Antony, M. B. (2011). Isolation, purification and identification of curcuminoids from turmeric (Curcuma longa L.) by column chromatography. Journal of Experimental sciences, 2(7).

38. Sahne, F., Mohammadi, M., Najafpour, G. D., & Moghadamnia, A. A. (2016). Extraction of bioactive compound curcumin from turmeric (Curcuma longa L.) via different routes: A comparative study. Pakistan Journal of Biotechnology, 13(3), 173-180.

39. Bener, M. (2019). Modeling and optimizing microwave-assisted extraction of antioxidants from Thymbra Spicata L. and characterization of their phenolic constituents. Food science and biotechnology, 28(6), 1733-1745.

40. Alupului, A., Calinescu, I., & Lavric, V. (2012). Microwave extraction of active principles from medicinal plants. UPB Science Bulletin, Series B, 74(2), 129-142.

41. Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrasonics sonochemistry, 34, 540-560.

42. Gupta SC, Prasad S, Kim JH, Patchva S, Webb LJ, Priyadarsini KI, et al., (2011). Multitargeting by curcumin as revealed by molecular interaction studies. Nat Prod Rep; 28:1937-1955.

43. Priyadarsini KI, (2013). Chemical and structural features influencing the biological activity of curcumin. Curr Pharm Des; 19: 2093-2100.

44. Newman J., Cragg M., Sanader M. (2013). Natural products as sources of new drugs over the period 1981–2002. J. Nat. Prod. 66, 1022–1037.

45. Sahoo K., Roy A., Chainy B. (2008). Protective effects of vitamin E and curcumin on l-thyroxine-induced rat testicular oxidative stress. ChemBiol Interact. 176, 121-128.

46. Yanagisawa D, Shirai N, Amatsubo T, Taguchi H, Hirao K, Urushitani M, et al., (2010). Relationship between the tautomeric structures of curcumin derivatives and their Aβ-binding activities in the context of therapies for Alzheimer's disease. Biomaterials; 31: 4179- 4185.

47. Khaligh, N. G., & Mihankhah, T. (2013). Aldol condensations of a variety of different aldehydes and ketones under ultrasonic irradiation using poly (N-vinylimidazole) as a new heterogeneous base catalyst under solvent-free conditions in a liquid-solid system. Chinese Journal of Catalysis, 34(12), 2167-2173.

48. Ngo, D. T., Tan, Q., Wang, B., & Resasco, D. E. (2019). Aldol condensation of cyclopentanone on hydrophobized MgO. promotional role of water and changes in the rate-limiting step upon organosilane functionalization. ACS Catalysis, 9(4), 2831-2841.

49. Pabon, H. J. J. (1964). A synthesis of curcumin and related compounds. Recueil des Travaux Chimiques des Pays-Bas, 83(4), 379-386.

50. Inayah, S. K., Pranowo, H. D., Wahyuningsih, T. D., & Anwar, C. (2019). Ultrasound-Assisted Synthesis of Some Curcumin Analogs and their Synergistic Effect with Ferulic Acid on α-Amylase Inhibition. In Materials Science Forum (Vol. 948, pp. 120-126). Trans Tech Publications Ltd.

51. GuptaC., Patchva S., AggarwalB. Therapeutic roles of curcumin: Lessons learned from clinical trials. AAPS Journal (2013) 15, 195–218.

52. Mahmood K., Zia K. M., Zuber M., Salman M., Anjum N. Recent developments incurcumin and curcumin based polymeric materials for biomedical applications: a review. Int J BiolMacromol(2015) 81, 877-890.

53. Somparn P., Phisalaphong C., Nakornchai S., Unchern S., Morales P. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. Biol Pharm Bull (2007) 30, 74–78.

54. Ahsan H., Parveen N., Khan U., Hadi M. Pro-oxidant, antioxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin. ChemBiol Interact (1999) 121, 161–175.

55. Sugiyama Y., Kawakishi S., Osawa T. Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. BiochemPharmacol (1996) 52, 519–525.

56. Anand P., Kunnumakkara B., Newman A., Aggarwal B. Mol. Pharm. (2007) 4, 807–818.

57. Shoba G., Joy D., Joseph T., Majeed M., Rajendran R., Srinivas P. Planta Med. (1998) 64, 353–356.

58. Bisht S., Feldmann G., Soni S., Ravi R.,Karikar C., Maitra A., Maitra A. J. Nanobiotechnol. (2007) 5, 3.

59. Li L., BraitehS., Kurzrock R. Cancer (2005) 104, 1322–1331.

60. Ma Z., Shayeganpour A., Brocks R., Lavasanifar A., Samuel J. Biomed. Chromatogr. (2007) 21, 546–552.

61. Several compounds with modified curcumin structures were created over the course of the last few decades, and it was discovered that these provided improved biological activity.

62. Bairwa K., Grover J., Kania M., Jachak M. RSC Adv. (2014) 4, 13946–13978.

63. Youssef D., Nichols E., Cameron S.. Design, synthesis, and cytostatic activity of novel cyclic curcumin analogues. Bioorg Med Chem Lett (2007) 17, 5624–5629.

64. Mosley A., Liotta C., Snyder P. Highly active anticancer curcumin analogues. Adv Exp Med Biol (2007) 595, 77–103.

65. Kühlwein F., Polborn K., Beck W., Anorg Z. Allg. Chem. (1997) 623, 1211–1219.

66. Leung M., Harada T., Kee W. Pharm. Des. (2013) 19, 2070–2083.

67. Tyagi P., Singh M., Kumari H., Kumari A., Mukhopadhyay K., Bactericidal Activity of Curcumin I Is Associated with Damaging of Bacterial Membrane, PLOS ONE, 10(3), P 1-16, (2015).

68. Ribeiro PD, Pavarina AC, Dovigo LN, Bruneti IL, Bagnato VS, Vergani CE, et al., (2013). Phototoxic effect of curcumin on methicillinresistant Staphylococcus aureus and L929 fibroblasts. Laser Med Sci; 28(2): 391-398.

69. Wang J, Zhou X, Li W, Deng X, Deng Y and Niu X, (2016). Curcumin protects mice from Staphylococcus aureus pneumonia by interfering with the self-assembly process of α-hemolysin. Sci Rep; 6: 28254.

70. Mun SH, Joung DK, Kim YS. Kang OH, Kim SB, Seo YS, et al., (2013). Synergistic antibacterial effect of curcumin against methicillinresistant Staphylococcus aureus. Phytomedicine; 20: 714-718.

71. Gunes H, Gulen D, Mutlu R, Gumus A, Tas T and Topkaya AE, (2016). Antibacterial effects of curcumin: An in vitro minimum inhibitory concentration study. Toxicol Ind Health; 32(2):246-250.

72. Singh A, Singh JV, Rana A, Bhagat K, Gulati HK, Kumar R, et al., (2019). Monocarbonyl curcumin-based molecular hybrids as potent antibacterial agents. ACS Omega; 4: 11673-11684.

73. Thomachan S, Sindhu S and John VD, (2016). Synthesis, characterization, antibacterial, antifungal and cytotoxic activity of curcuminoid analogues with trisubstituted phenyl and anthracenyl ring and their zinc (II), copper (II) and vanadyl (IV) chelates. IJPC; 6(3): 78- 86.

74. Saleheen D, Ali SA, Ashfaq K, Siddiqui AA, Agha A and Yasinzai MM, (2002). Latent activity of curcumin against Leishmaniasis in vitro. Biol Pharm Bull; 25: 386-389.

75. Perez-Arriaga L, Mendoza-Magana ML, Cortes-Zarate R, Corona-Rivera A, Bobadilla-Morales L, Troyo-Sanroman R, et al., (2006). Cytotoxic effect of curcumin on Giardia lamblia trophozoites. Acta Trop; 98: 152-161.

76. Cui L, Miao J and Cui L, (2007). Cytotoxic effect of curcumin on malaria parasite Plasmodium falciparum: Inhibition of histone acetylation and generation of reactive oxygen species. Antimicrob Agents Chemother; 51(2): 488-494.

77. Nandakumar DN, Nagaraj VA, Vathsala PG, Rangarajan P and Padmanaban G, (2006). Curcumin-artemisinin combination therapy for malaria. Antimicrob Agents Chemother; 50: 1859-1860.

78. Silvaa CC, Pachecoa BS, Nevesb RS, Alvesb MS, Sena-Lopesb A, Mourac S, et al., (2019). Antiparasitic activity of synthetic curcumin monocarbonyl analogues against Trichomonas vaginalis. Biomed Pharmacother; 111: 367-377.

79. Goindi S and Kaur IP, (2008). Antimutagenicity of curcumin related compounds against genotoxic heterocyclic amines from cooked food. The structural requirement. Food Chem; 111(3): 573-579.

80. Liang L, Xue YB, Liu Z, Peng SS, He Y, Zhang Y et al., (2015). Coumarin derivatives from Ainsliae fragrance and their anticoagulant activity. Sci Rep; 5: 13544.

81. Al-Hujaily EM, Mohamed AG, Al-Sharif I, Youssef KM, Manogaran PS, Al-Otaibi B, et al., (2011). PAC, a novel curcumin analogue, has anti-breast cancer properties with higher efficiency on ERnegative cells. Breast Cancer Res Treat; 128: 97-107.

82. Shishodia S, Potdar P, Gairola CG and Aggarwal BB, (2003). Curcumin (diferuloylmethane) down-regulates cigarette smoke-induced NF-kB activation through inhibition of IkBa kinase in human lung epithelial cells: correlation with suppression of COX-2, MMP-9 and cyclin D1. Carcinogenesis; 24(7): 1269-1279.

83. Tsai JR, Liu PL, Chen YH, Chou SH, Cheng YJ, Hwang JJ, et al., (2015). Curcumin inhibits non-small cell lung cancer cells metastasis through the adiponectin/NF-κb/MMPs signaling pathway. PLoS ONE 10(12): e0144462. https://doi:10.1371/journal.pone e0144462.

84. Wu J, Cai Z, Wei X, Chen M, Ying S, Shi L, et al., (2015). Antilung cancer activity of the curcumin analog JZ534 in vitro. Biomed Res Int; Article ID 504529. http://doi:10.5155/2015/504529.

85. Montazeri M, Slotanahmadi P, Mohaghegh M, Panahi A, Khodi S, Zarghami N, et al., (2017). Antiproliferative and apoptotic effect of dendrosomal curcumin nanoformulation in P53 mutant and wide-type cancer cell lines. Anticancer Agents Med Chem; 17 (5): 662-673.

86. Singh M and Singh N, (2011). Curcumin counteracts the proliferative effect of estradiol and induces apoptosis in cervical cancer cells. Mol Cell Biochem; 347 (1-2); 1-11.

87. Rao VE and Sudheer P, (2011). Revisiting curcumin chemistry part I: A new strategy for the synthesis of curcuminoids. Indian J Pharm Sci; 73: 262-270.

88. Garel L and Saint-Jalmes L, (2006). One-pot fluoro-dediazoniation of anilines in organic medium. Tetrahedron Lett; 47: 5705- 5708.

89. Johnson JJ and Mukhtar H, (2007). Curcumin for chemoprevention of colon cancer. Cancer Lett; 255(2): 170-181.

90. Rajitha B, Belalcazar A, Nagaraju GP, Shaib WL, Snyder JP, Shoji M, et al., (2016). Inhibition of NF-κB translocation by curcumin analogs induces G0/G1 arrest and downregulates thymidylate synthase in colorectal cancer. Cancer Lett; 373(2): 227-233.

91. Sankar P, Telang AG, Kalaivanan R, Karunakaran V, Manikam K and Sarkar SN, (2013). Effect of nanoparticle- encapsulated curcumin on arsenic induce liver toxicity in rats. Environ Toxicol; 30: 628-637.

92. Aftab N and Vieira A, (2010). Antioxidant activities of curcumin and combination of this curcuminoid with other phytochemicals. Phytother Res; 24(4): 500-502.

93. Shang YJ, Jin XL, Shang XL, Tang JJ, Liu GY, Dai F, et al., (2010). Antioxidant capacity of curcumin-directed analogues: Structureactivity relationship and influence of microenvironment. Food Chem; 119: 1435-1442.

94. Hatcher H, Planalp R, Cho J, Torti FM and Torti SV, (2008). Curcumin: From ancient medicine to current clinical trials. Cell Mol Life Sci; 65(11): 1631-1652.

95. He Y, Yue Y, Zheng X, Zhang K, Chen S and Du Z, (2015). Curcumin, inflammation, and chronic diseases: How are they linked? Molecules; 20: 9183-9213.

96. Gao X, Kuo J, Jiang H, Deep D, Liu Y and Diving G, (2004). Immuno-modulatory activity of curcumin: Suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production in vitro. Biochem Pharmacol; 68(1): 51-61.

97. Surh YJ, (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. Food Chem Toxicol; 40(8): 1091-1097

98. Paulino N, Paulino AS, Diniz SN, Mendonça S, Gonçalves ID, Flores FF et al., (2016). Evaluation of the anti-inflammatory action of curcumin analog (DM1): Effect on iNOS and COX-2 gene expression and autophagy pathways.Bioorg Med Chem; 24: 1927-1935.

99. Alka, C.; Lynn, M. N.; Itxaso, M.; Alan, D.W.D.; Dilip, K.R.; Current Status and Future Prospects of Marine Natural Products (MNPs) as Antimicrobials Marine Drugs 2017, 15(9), 272.

100. Hamed, O.; Mehdawi, N.; Taha, A.; Hamed, E.; Al-Nuri, M.; Hussein, A.; Synthesis and Antibacterial Activity of Novel Curcumin Derivatives Containing Heterocyclic Moiety. *Iranian Journal of Pharmaceutical Research*, 2013, *12(1)*, 47-56.

101. Panawala, L. (2017). Difference between gram positive and gram negative bacteria. Epedıaa, 3, 1-13.

102. Alkasher, I., & Badi, W. (2020). Gram-positive vs gram-negative.

103. Bengoechea, J. A., & Sa Pessoa, J. (2019). Klebsiella pneumoniae infection biology: living to counteract host defences. FEMS microbiology reviews, 43(2), 123-144.

104. Klebsiella pneumoniae in Healthcare Settings, Centers for Disease.

105. Braz, V. S., Melchior, K., & Moreira, C. G. (2020). Escherichia coli as a multifaceted pathogenic and versatile bacterium. Frontiers in Cellular and Infection Microbiology, 10, 548492.

106. Kabiru L.M., Bello M., Kabir J., Grande L., Morabito S., Detection of Pathogenic Escherichia coli in Samples Collected at an Abattoir in Zaria, Nigeria and at Different Points in the Surrounding Environment, Int. J. Environ. Res. Public Health, 12, P 679-691, (2015).

107. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews, 28(3), 603-661.

108. Green, B. N., Johnson, C. D., Egan, J. T., Rosenthal, M., Griffith, E. A., & Evans, M. W. (2012). Methicillin-resistant Staphylococcus aureus: an overview for manual therapists. Journal of chiropractic medicine, 11(1), 64-76.

109. Pruthi S., MRSA infection, Mayo Foundation for Medical Education and Research (MFMER), (2011).

110. Iwase, T., Uehara, Y., Shinji, H., Tajima, A., Seo, H., Takada, K., ... & Mizunoe, Y. (2010). Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature, 465(7296), 346-349.

111. Jumaa S., Karaman R., Antibiotics, In: Commonly Used Drugs, P. 1-35, (2015).

112. Etebu E., Arikekpar I., Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives, IJAMBR, 4, P. 90-10, (2016).

113. Etebu E., Arikekpar I., Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives, IJAMBR, 4, P 90-101, (2016).

114. Yin N., Ma W., Pei J., Ouyang Q., Tang C., Lai L., Synergistic and Antagonistic Drug Combinations Depend on Network Topology, PLOS ONE, 9(4), P1-7, (2014).

115. Atawodi S., Atawodi J.C. Dzikwi A.A., POLYMERASE CHAIN REACTION: THEORY, PRACTICE AND APPLICATION: A REVIEW,

116. Westermeier R., Gel Electrophoresis, Encyclopedia of life science, John Wiley and Sons, P1-7, (2005).

117. Lee P.Y., Costumbrado J., Hsu C.Y., Kim Y.H., Agarose Gel Electrophoresis for the Separation of DNA Fragments, Journal of Visualized Experiments, 62, P1-5, (2012).

118. Da Silva, C. M., da Silva, D. L., Modolo, L. V., Alves, R. B., de Resende, M. A., Martins, C. V., & de Fátima, Â. (2011). Schiff bases: A short review of their antimicrobial activities. Journal of Advanced research, 2(1), 1-8.

119. Phillips, D. H., & Arlt, V. M. (2009). Genotoxicity: damage to DNA and its consequences. Molecular, clinical and environmental toxicology, 87-110.

120. SAKS M., Upreti S., Rajendra S.V., Dang R., Genotoxicity: Mechanisms, Testing Guidelines and Methods, Global journal of Pharmacy & pharmaceutical Science, 5, P1-6, (2017).

121. Savale S.K., Genotoxicity of Drugs: Introduction, Prediction and Evaluation, Asian Journal of Biomaterial Research, 4(6), P 1-29, (2018).

122. Andrews, J. M., BSAC standardized disc susceptibility testing method (version 5), Journal of Antimicrobial Chemotherapy,58(3), P 511-529, (2006).

123. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 28th ed. CLSI supplement. M100.

124. Adwan G, Adwan K, Jarrar N, Salameh Y. Prevalence of *seg*, *seh* and *sei* genes among clinical and nasal swab of *Staphylococcus aureus* isolates. Brittish Microbiology Research Journal, 2013; 3(2): 139-149

125. Atienzar, F., Evenden, A., Jha, A., Savva, D., & Depledge, M. (2000). Optimized RAPD analysis generates high-quality genomic DNA profiles at high annealing temperature. Biotechniques, 28(1), 52-54.

126. Abu-Hijleh A., Adwan G., Abdat W., Biochemical and molecular evaluation of the plant Ecballium elaterium extract effects on Escherichia coli. J Adv Biol Biotechnol, 19(2), P 1-11, (2018).

127. Atienzar F.A., Venier P., Jha A.N., Depledge, MH. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations, Mutat Res., 521(1-2), P 151-163, (2002).

128. Hamed O., Mehdawia N., Abu Taha A., Hamed E., Al-Nuri M., Hussein A. Synthesis and Antibacterial Activity of Novel Curcumin Derivatives Containing Heterocyclic Moiety.Iranian Journal of Pharmaceutical Research (2013) 12, 1, 47-56.

129. Kühlwein F., Polborn K., Beck W., Anorg Z. Allg. Chem. (1997) 623, 1211–1219.

130. Lalrotluanga, Kumar, S., Gurusubramanian, G. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage in mosquito larvae treated with plant extracts. Science Vision (2011) 11, 3, 155-158.

131. Ciğerci H., Cenkci S., Kargıoğlu M., Konuk M. Genotoxicity of Thermopsisturcica on Allium cepa L. roots revealed by alkaline comet and random amplified polymorphic DNA assays. Cytotechnology (2016) 68,4, 829-838.

132. Hajar S., Gumgumjee M. Antimicrobial activities and evaluation of genetic effects of Moringa peregrina (forsk) fiori using molecular techniques. International Journal of Plant and Animal Environmental Sciences (2014) 4, 1, 65-72.

133. Mahmood K., Zia K. M., Zuber M., Salman M., Anjum N. Recent developments incurcumin and curcumin based polymeric materials for biomedical applications: a review. Int J BiolMacromol (2015) 81, 877-890.

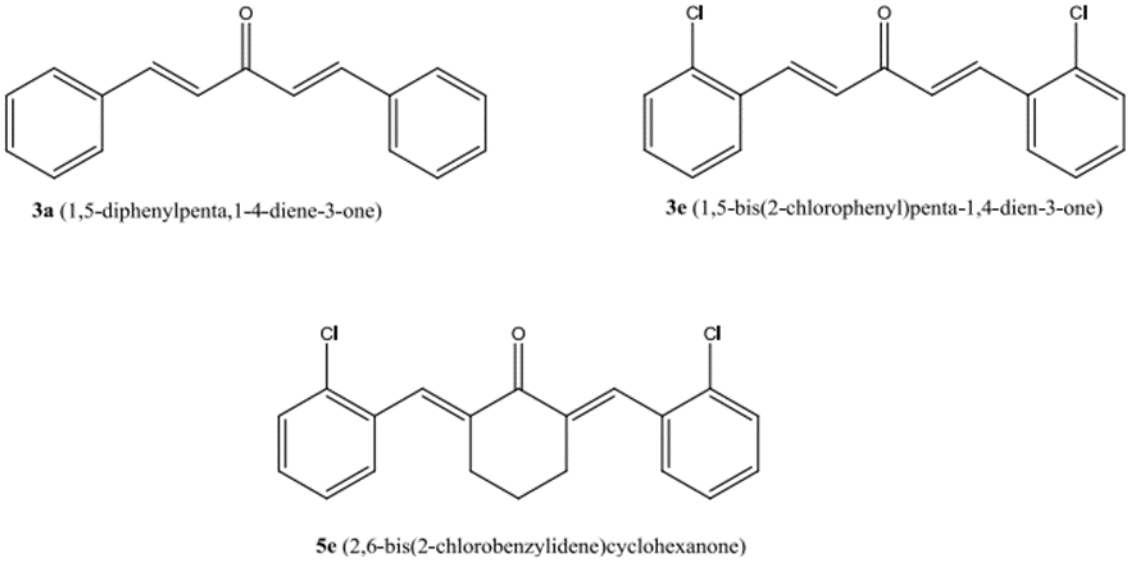
Appendices

Appendix A

Figures

Figure 1.11

Curcumin analogues with ant trichomoniasis activity



3a (1,5-diphenylpenta,1-4-diene-3-one)

3e (1,5-bis(2-chlorophenyl)penta-1,4-dien-3-one)

5e (2,6-bis(2-chlorobenzylidene)cyclohexanone)

Figure 1.12

Curcumin analogues with ant breast cancer activity

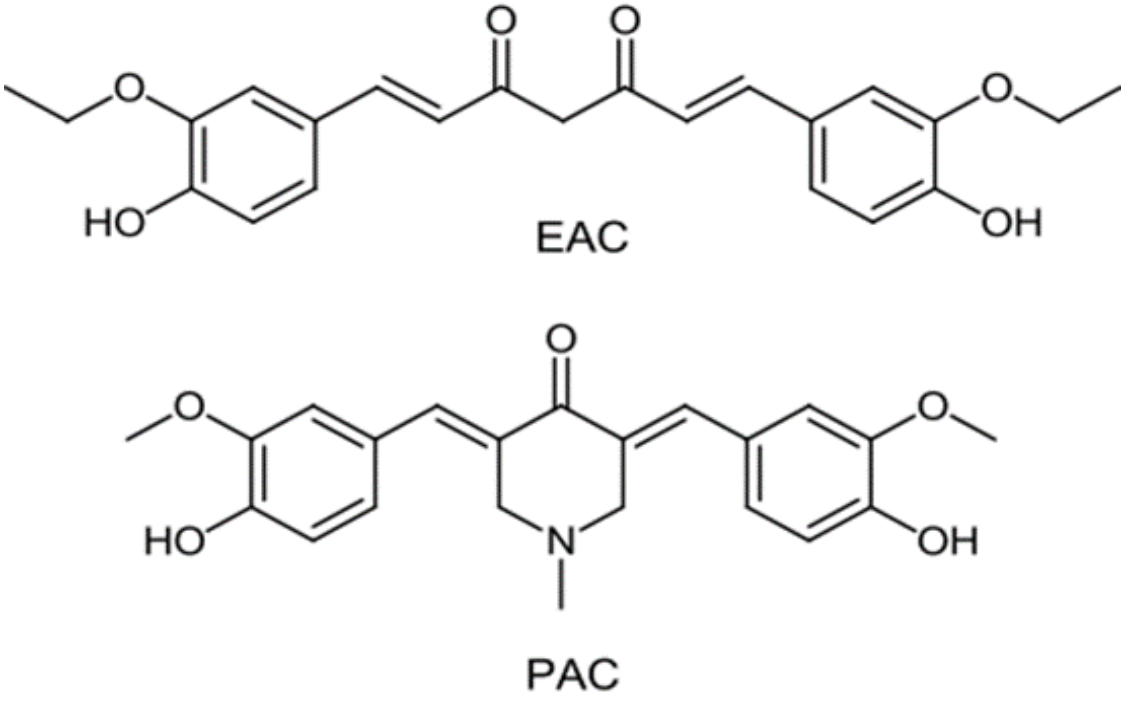


Figure 1.13

The curcumin analogue JZ534 with anti-lung cancer efficacy

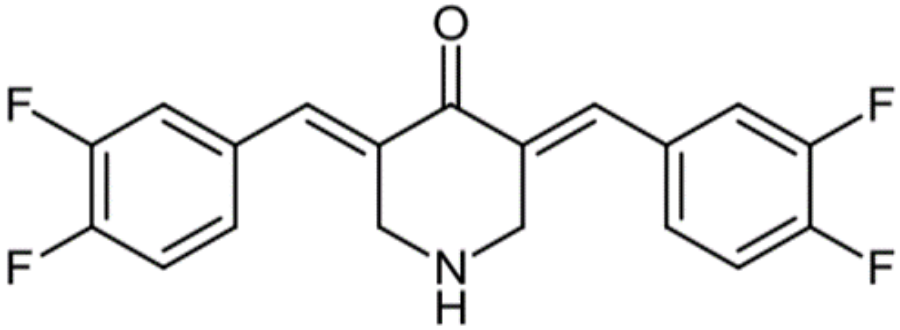
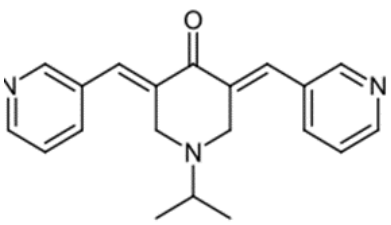
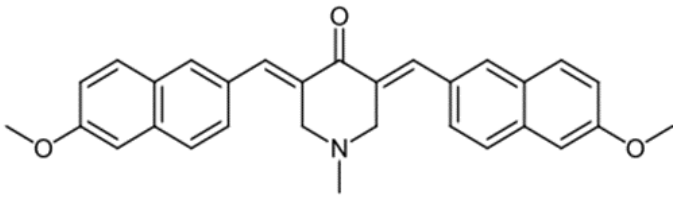


Figure 1.14

Curcumin analogues RL118 and RL121 with activity against prostatic cancer



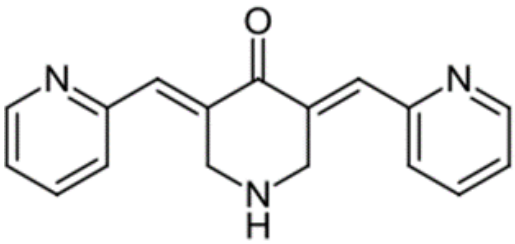
RL118



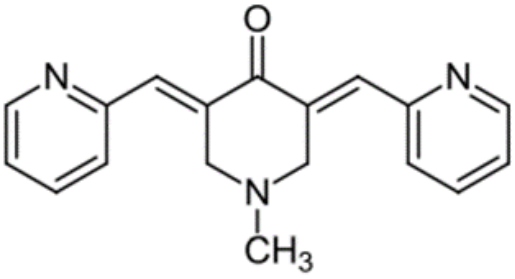
RL121

Figure 1.15

The curcumin analogues EF31 and UBS 109 with activity against colorectal cancer



EF31



UBS 109

Figure 1.16

A number of curcumin analogues with antioxidant activity

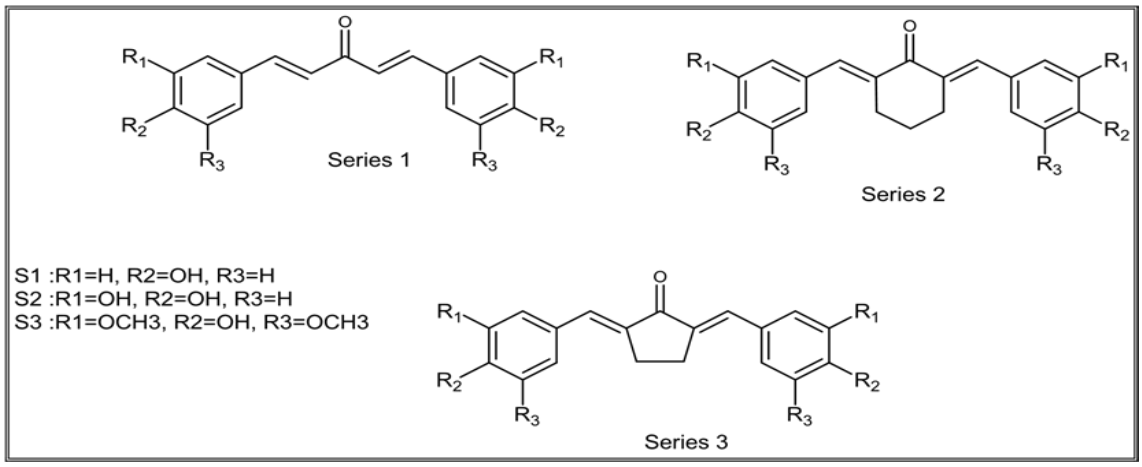


Figure 1.17

Curcumin analogue DM1

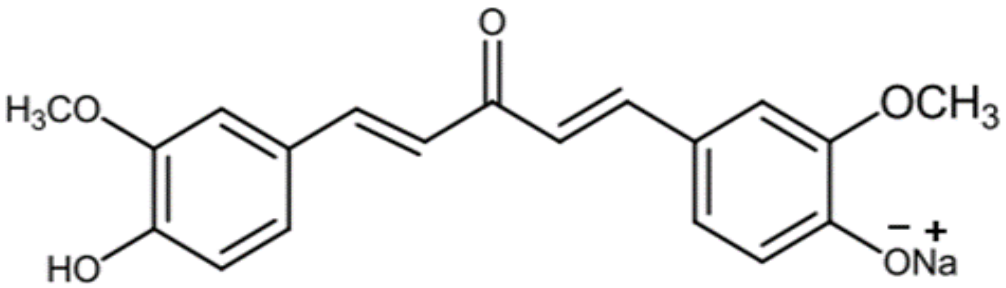


Figure 1.18

Comparison between Gram negative bacteria

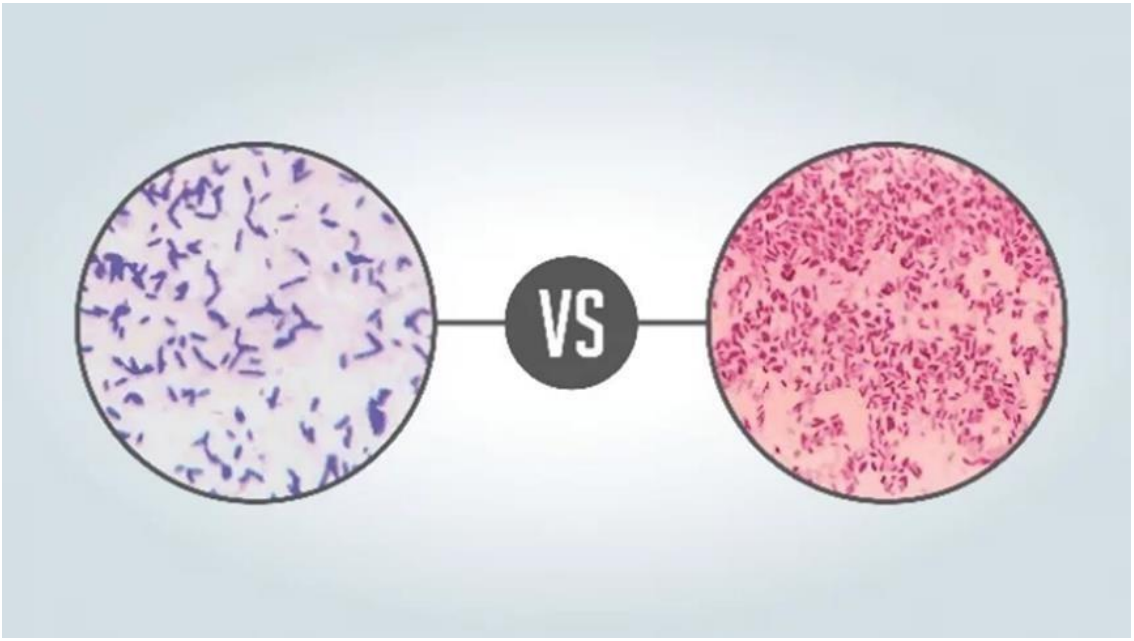


Figure 1.19

Klebsiella pneumoniae is a type of Gram-negative bacteria

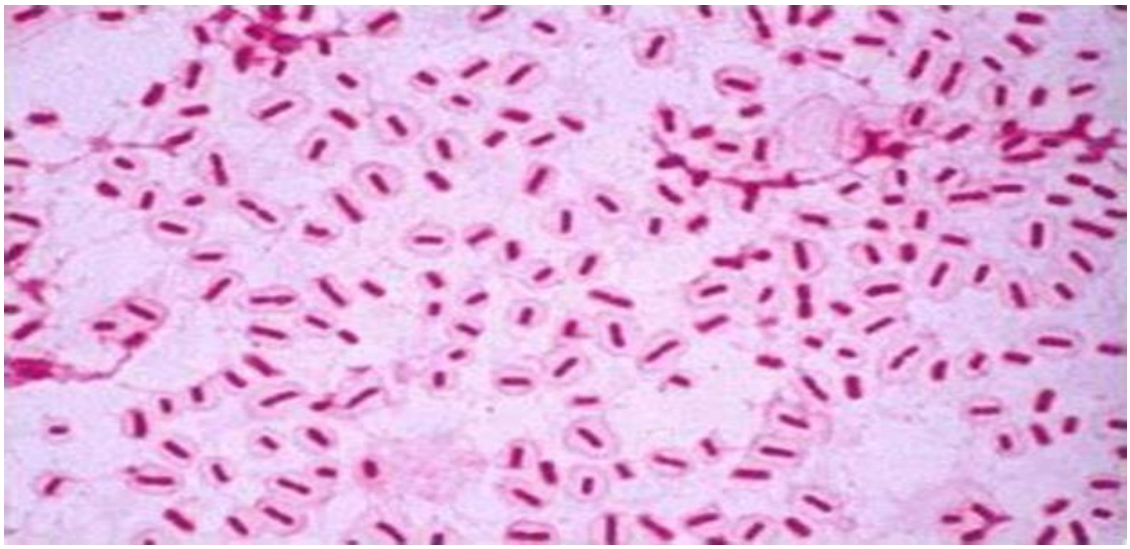


Figure 1.20

Escherichia coli is a gram negative bacteria

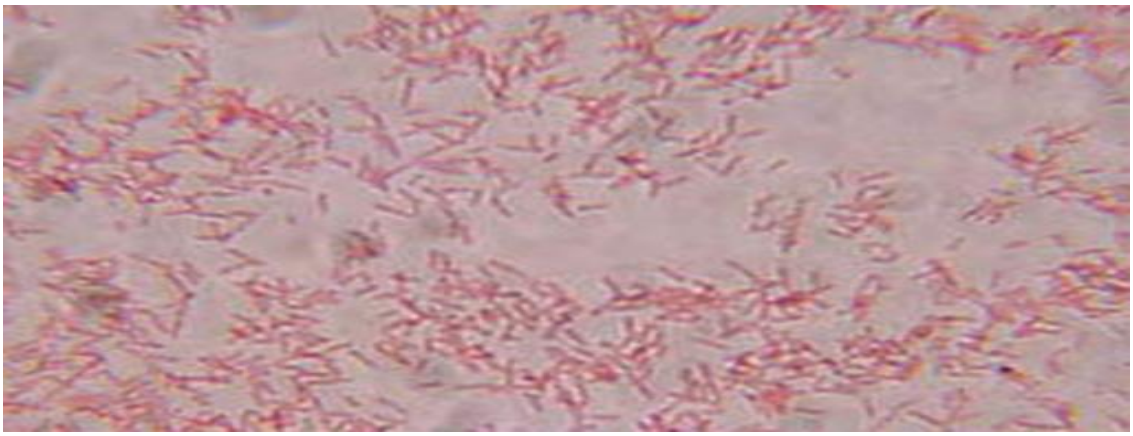


Figure 1.21

Staphylococcus aureus and (MRSA) and epidermidis a gram positive bacteria

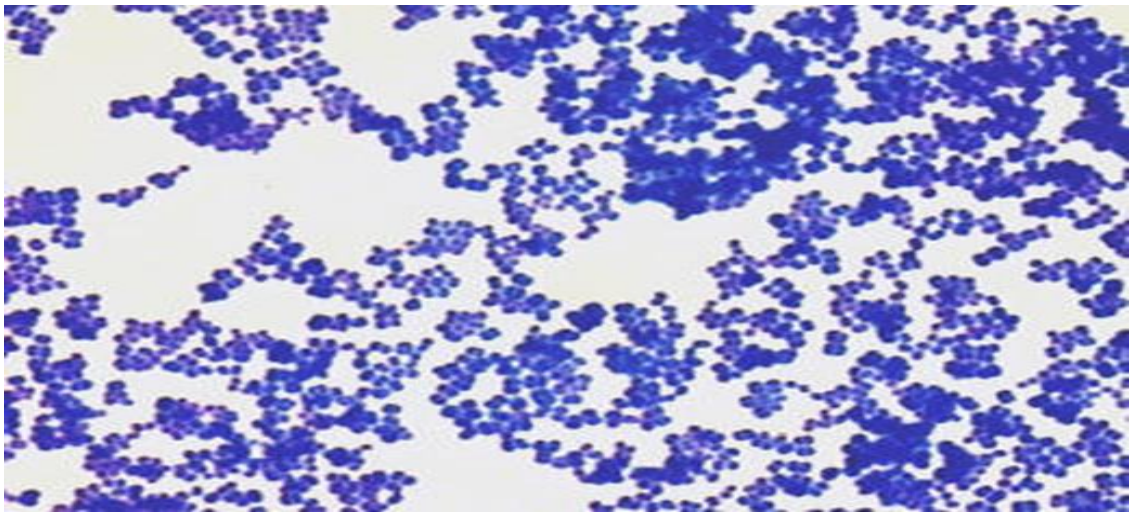


Figure 3.1

FT-IR of Schiff base 2

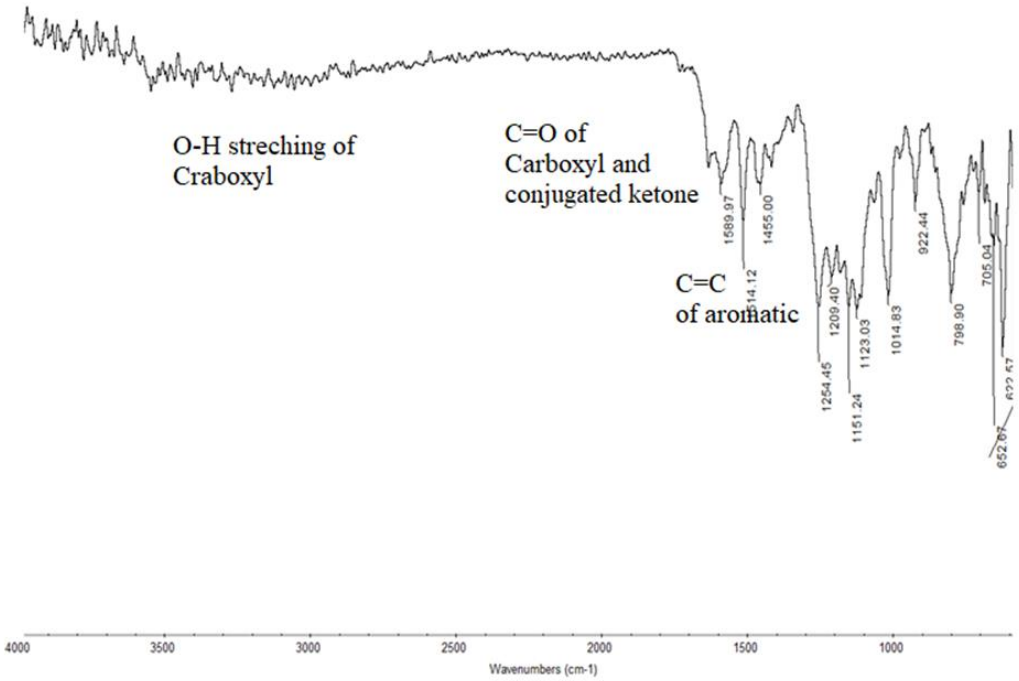


Figure 3.2

FT-IR of Schiff base 3

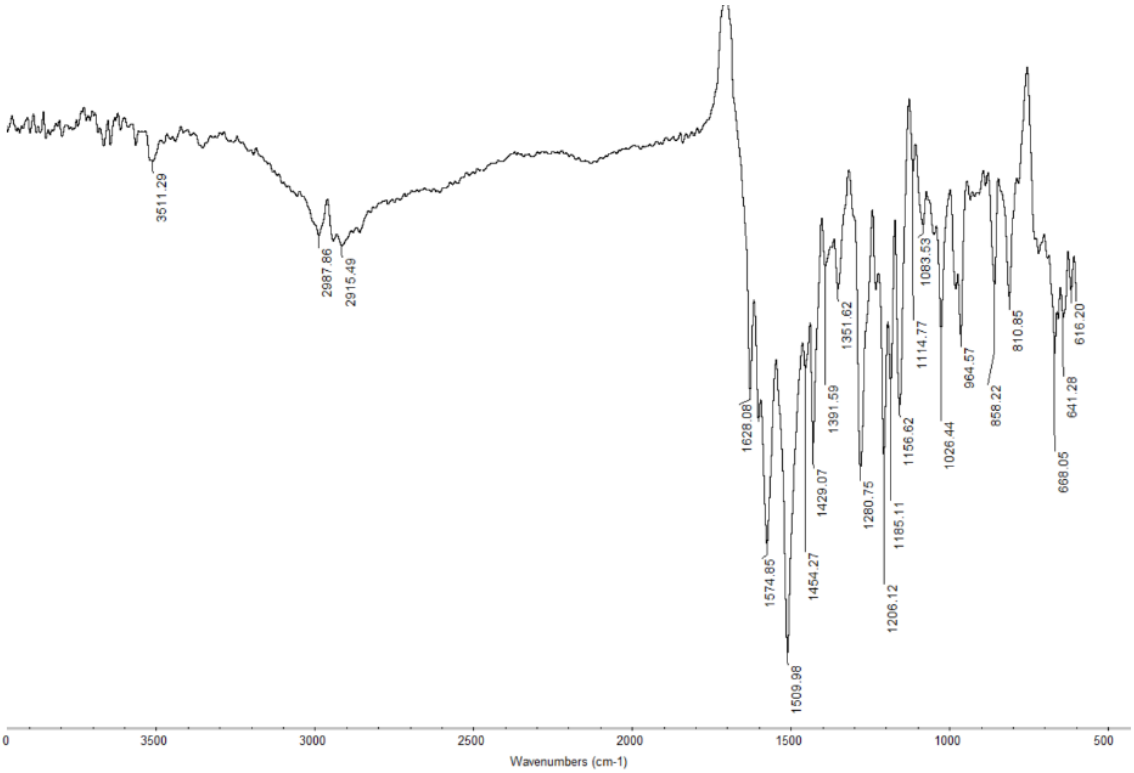


Figure 3.3

FT-IR of Schiff base 4

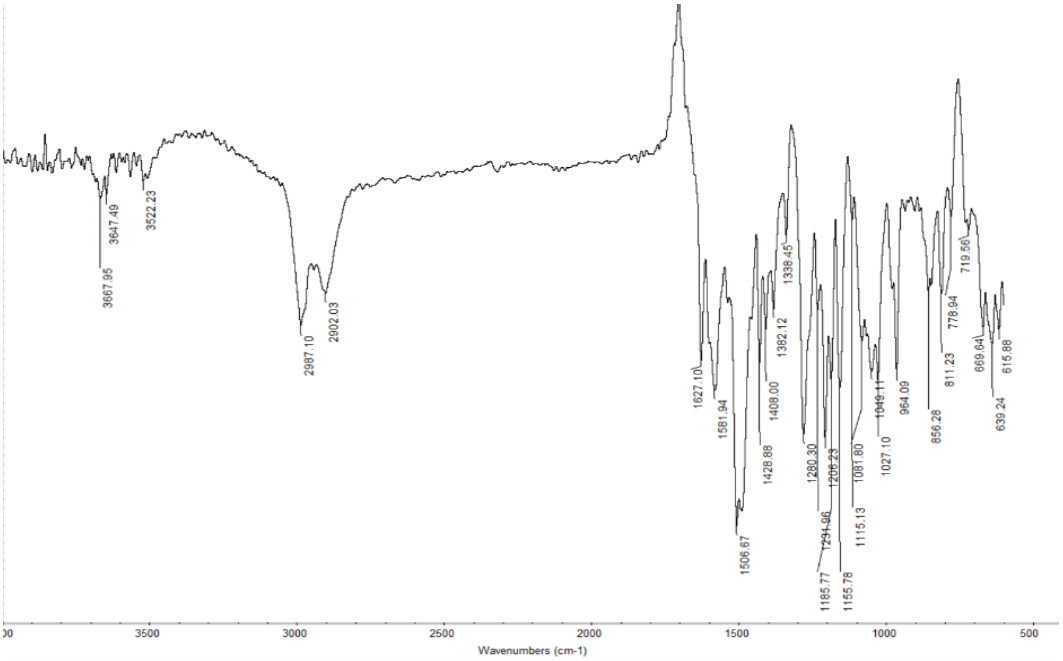


Figure 3. 4

FT-IR of Schiff base 5

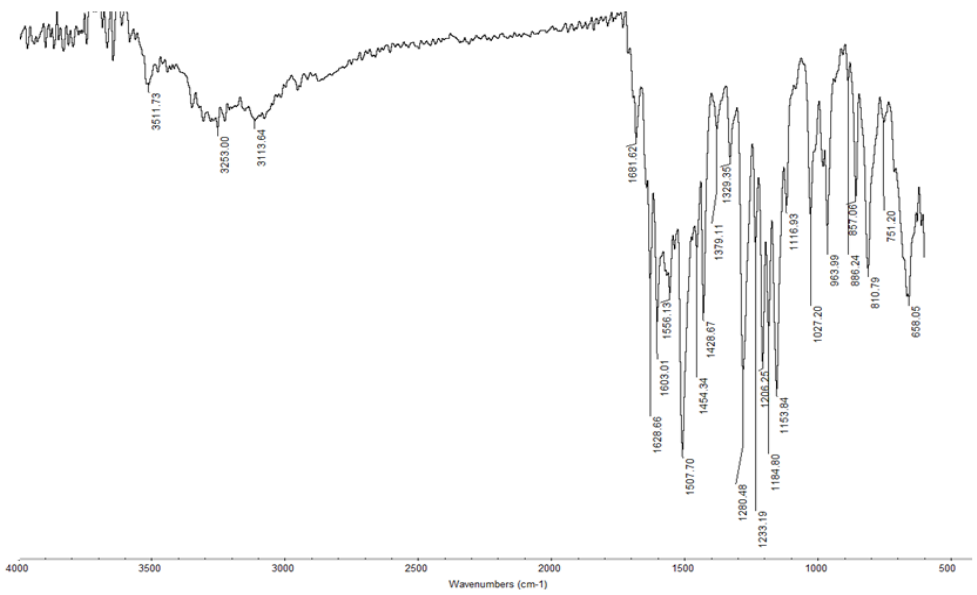
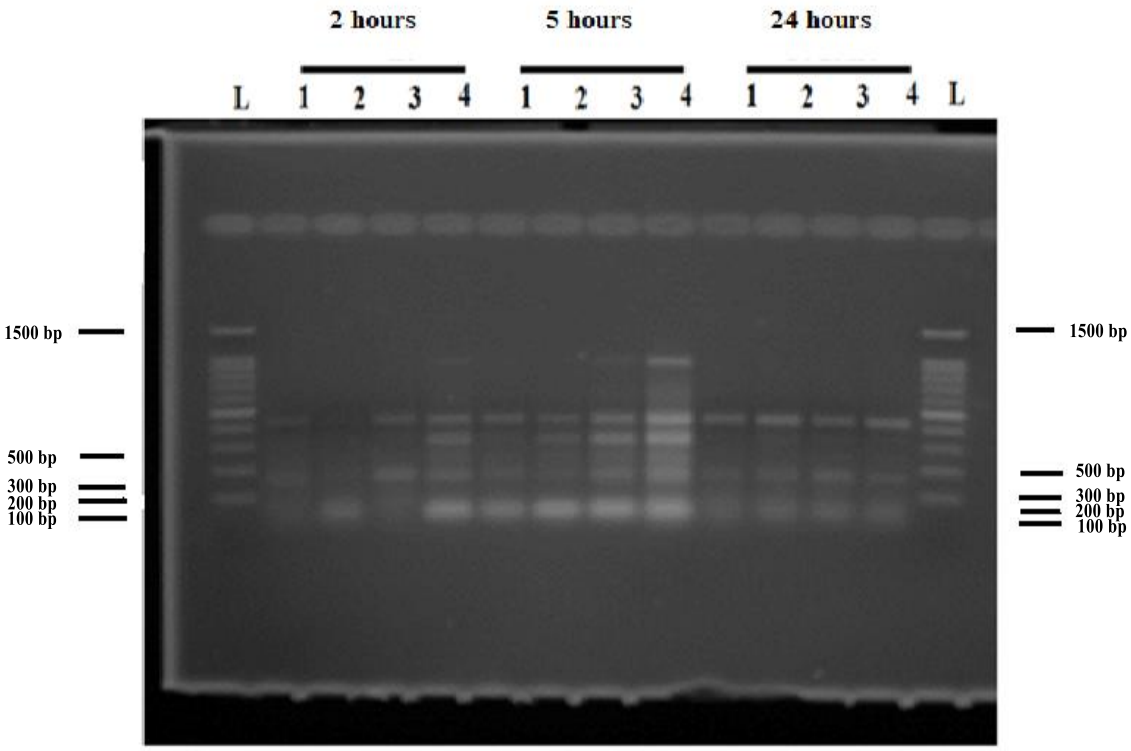


Figure 3. 5

ERIC-PCR profile of E. coli strain treated and untreated with different concentrations of compound 5



جامعة النجاح الوطنية

كلية الدراسات العليا

الكيركومين مع وظائف قاعدة شيف: الأنشطة التوليفية والمضادة للبكتيريا

إعداد

رانية عبد اللطيف

إشراف

د. عثمان حامد

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس –فلسطين.

2023

الكيركومين مع وظائف قاعدة شيف: الأنشطة التوليفية والمضادة للبكتيريا

إعداد

رانية عبد اللطيف

إشراف

د. عثمان حامد

الملخص

الكيركومين مادة طبيعية لها مجموعة واسعة من الوظائف البيولوجية ومصدر للعديد من الأدوية. من الصعب إنتاج عقاقير طبيعية يمكن أن تمنع مقاومة البكتيريا للمضادات الحيوية. لتحقيق هذا الهدف، تم تحضير قواعد شيف من خلال عملية وعاء واحد تتضمن تفاعل الكيركومين مع الأحماض الأمينية المختلفة.

كانت الأحماض الأمينية المختارة لهذا العمل هي تلك ذات الوظائف الحلقية غير المتجانسة بالإضافة إلى مجموعات الأمين والمجموعات الكربوكسيلية. وهذه الأحماض الأمينية المختارة كانت هيستيدين، ليسين، سيستين، وأرجينين. تم اختبار هياكل المركبات المنتجة بواسطة FT-IR و H NMR والتحليل الطيفي الكتلي والنشاط المضاد للبكتيريا.

تم اختبار قواعد شيف ضد البكتيريا موجبة الجرام وهي Staphylococcus aureus و Staphylococcus epidermidis و Staphylococcus aureus المقاومة للميثيسيلين. والبكتيريا سالبة الجرام وهي Escherichia coli و Klebsiella pneumonia.

كانت قاعدة شيف 5 التي تنتج من تفاعل الكيركومين مع الأرجينين هي أكثر قيمة MIC فاعلية بمقدار 200 ميكروغرام مل. قاعدة شيف 2 التي تنتج من تفاعل الكيركومين مع الهيستيدين قامت

بتغيير الحمض النووي لسلالة الإشريكية القولونية، ويمكن أن يعزى ذلك إلى وجود العديد من ذرات النيتروجين في بنية المركب.

الكلمات المفتاحية: قواعد شيف، مادة طبيعية، الكيركومين، أحماض أمينية.