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Thème

Study of the synergistic effect of 6-APA nucleus with the salicylaldehyde on some bacteria

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Finally, I leave you with these immortal words, and wish all the best for the rest of my colleague students.

"A man would do nothing if he waited until he could do it so well that no one would find fault with what he has done".

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General Introduction

General introduction

The success of the modern anti-infective therapy, are the consequences of extensive research, they have been developed in the areas of synthesis, analysis and control of pharmaceuticals.

One of the applications of chemical analysis methods was essential for the identification of components of drug molecules including antibiotics.

The chemistry of semi-synthetic penicillins begins to develop after the discovery of the acid 6 - aminopenicillanic (6-APA) in 1950 and after obtaining the pure crystal in 1959. Starting material in the synthesis of penicillin, has unlimited properties. It can be acylated with different acylating agents, obtaining derivatives, which differ in their spectrum of activity [1].

The main of this work is an attempt to fight against antibiotics resistance which of course involves the test for new antibiotics. In this regard, we will try to test salicyaldehyde, which was one of the first antipyretic (fever medicine fighter) on three types of bacteria, and the study of its synergic effect with 6-aminopécillanic acid nucleus.

This work is consisting of five chapters; the first chapter to represents the generality of aromatics compounds, while the second chapter represents generality of antibiotics, the third chapter describes the general microbiology. The fourth chapter presents the methods and materials used, and at the fifth results and discussions, and conclusion.

Chapter I :
Aromatic compounds

I. Aromatic compounds

I.1. Background for Aromatic Compounds

Kekule proposed the structure of benzene in 1865. The carbons in benzene are sp^2 hybridized, have a bond angle of 120^o, and have a trigonal planar shape. Benzene can be drawn with all carbons and hydrogens, with just lines, or using a circle in the ring.

π electrons are counted as follows: each double bond counts as two p electrons, each + charge counts as zero π electrons, each B charge counts as two π electrons, each radical counts as one π electron. Set the total number of π electrons as equal to 4n + 2 (Huckel's Rule) solve for n. If the compound is aromatic, then it is cyclic with a continuous ring of overlapping π orbitals and n equals a whole number. If the compound is antiaromatic, then it is cyclic with a continuous ring of overlapping p orbitals, but n does not equal a whole number. If the compound is not aromatic, then it is noncyclic or is cyclic without a continuous ring of overlapping p orbitals [2].

I.2. Definition

The aromatic compounds contain at least one benzene ring, a ring of six carbons atoms with alternate double and single bonds. Aromatic compounds are called so because many of them possess a fragrant smell [2].

Typical examples of aromatic compounds are given below:

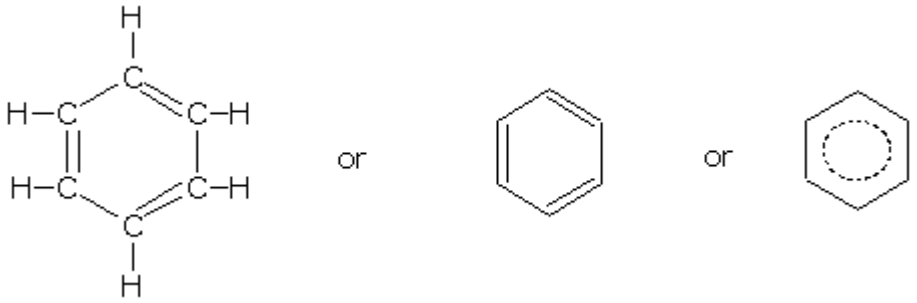


Figure I. 1 Structure of Benzene

The aromatic compounds may have a side-chain or a functional group attached directly to the ring. For example:

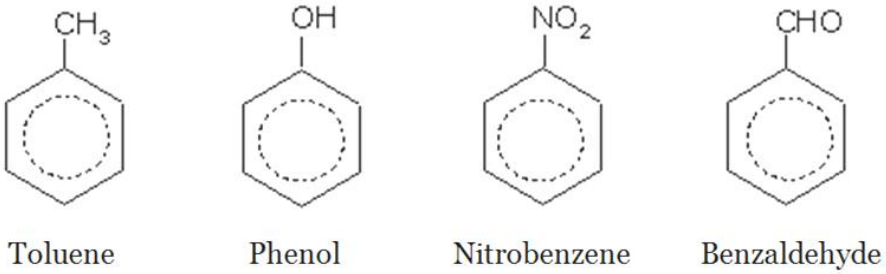


Figure I. 2 Some aromatic compounds with a side-chain or functional group

The aromatic compounds may also contain more than one benzene ring fused together

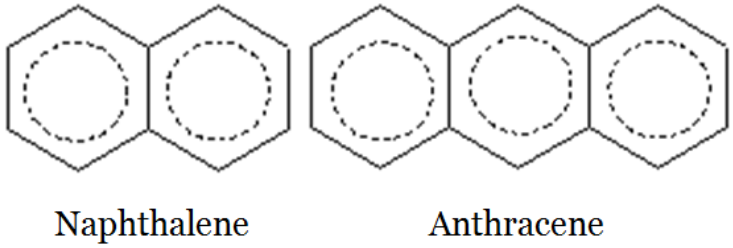


Figure I. 3 Aromatic compounds have more than one benzene ring

There are two classifications of aromatic compounds.

I.2.1. Nucleus Substituted Compounds

When the functional group or any substituent, in aromatic compounds is directly attached to the benzene ring, it is called nucleus substituted compound. Such compounds are named as the derivatives of benzene under the IUPAC system. However, the common names of many such compounds are retained by IUPAC [2].

I.2.2. Side-chain Substituted Compounds

Aromatic compounds where the functional group is present in the side-chain of the ring are called side-chain substituted compounds. Side-chain substituted compounds are named as the phenyl derivatives of the corresponding aliphatic compounds [2].

I.2.3. Nomenclature of Aromatic Compounds

$C_6H_5CH_3$, C_6H_5OH, $C_6H_5NH_2$, $C_6H_5OCH_3$, are named respectively Toluene. Phenol, Aniline, Anisole, and Styrene. $C_6H_5CH=CH_2$ Benzene with an Aldehyde group is named Benz Aldehyde, with a methyl ketone group is called Acetophenone, and with a carboxylic acid group is named Benzoic Acid. So, the Benzene is the parent compound. Number the ring so that the groups have the lowest possible numbers. If a special name is used for a Benzene derivative, then that group is in position one; then number the other groups. Or the means that the two groups are on adjacent carbons or on the 1, 2 positions. Meta means that the two groups are separated by one carbon or on the 1,3-positions. Para means that the two groups are on opposite ends or on the 1, 4-positions. The fused ring structures of Anthracene, Naphthalene, and Phenanthrene are drawn out [2].

Common Names of Some Aromatic Compounds

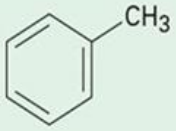
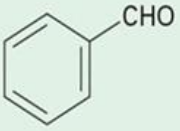
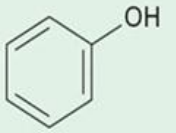
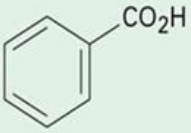
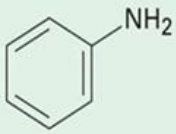
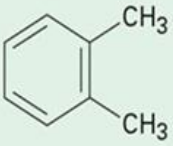
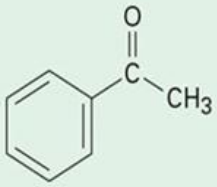
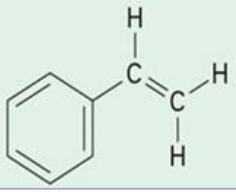
Structure	Name	Structure	Name
	Toluene (bp 111 °C)		Benzaldehyde (bp 178 °C)
	Phenol (mp 43 °C)		Benzoic acid (mp 122 °C)
	Aniline (bp 184 °C)		*ortho*-Xylene (bp 144 °C)
	Acetophenone (mp 21 °C)		Styrene (bp 145 °C)

Figure I. 4 Common names of some aromatic compounds

- **Monosubstituted Benzenes**

  - Systematically named in the same manner as the other hydrocarbons "benzene" used as parent name:
  - C_6H_5Br is Bromobenzene;
  - $C_6H_5NO_2$ is Nitrobenzene;
  - $C_6H_5CH_2CH_2CH_3$ is Propylbenzene.

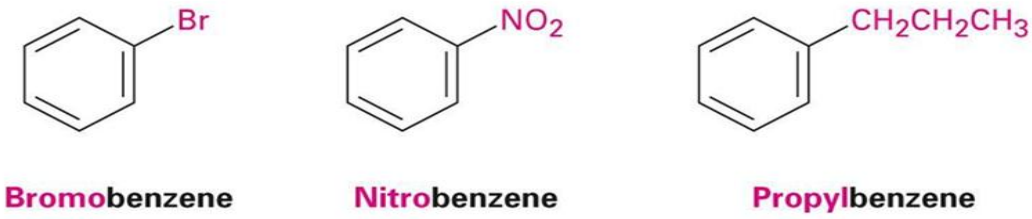


Figure I. 5 Some monosubstituted Benzenes

- **Arenes**

  - Alkyl-substitutedbenzenes;
  - Named depending on the size of the alkyl group ;
  - Alkyl substituent smaller than the ring (6 or fewer carbons), named as an alkyl substituted benzene ;
  - Alkyl substituent larger than the ring (7 or more carbons), named as a phenyl-substituted alkane [2].

- **Phenyl**

  - Derived from the Greek phenol ("I bear light") ;
  - Michael Faraday discovered benzene in 1825 from the oily residue left by illuminating gas used in London street lamps ;
  - Used for the $-C_6H_5$ unit when the benzene ring is considered as a substituent ;
  - Abbreviated as Ph. or F (Greek phi) [2].

- **Benzyl**

  - Used for the $C_6H_5CH_2$- group ;

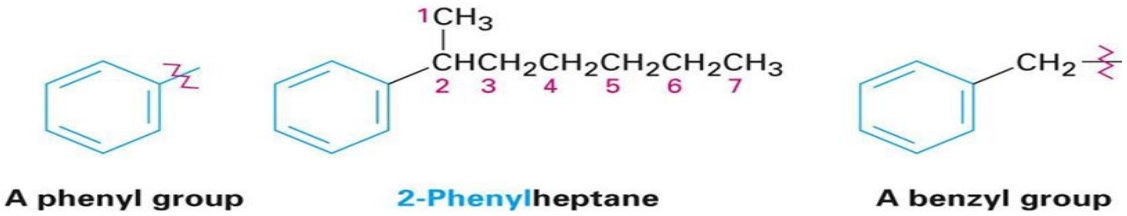


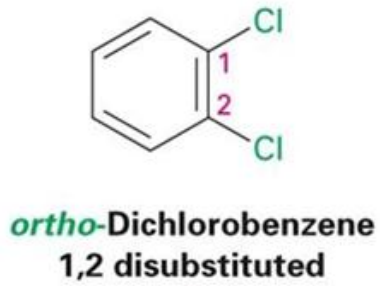
Figure I. 6 Some aromatic compounds have benzyl group

- **Disubstituted benzenes**

  - Named using one of the prefixes;

- 1-**Ortho- (o-)**

- Ortho-disubstituted benzene has two substituents in a 1,2 relationship.

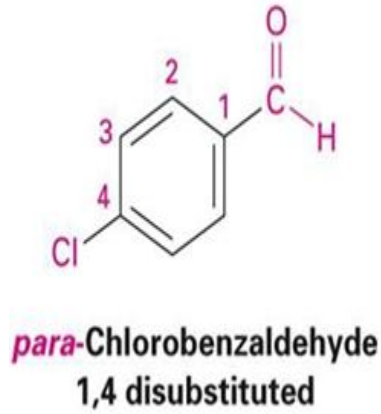


- **2. Meta- (m-)**

- Meta-substituted benzene has. Its substituents7 in a 1, 3 relationships.

- **3- Para-(p)**

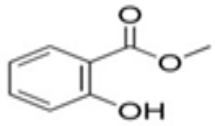
- Para-disubstituted benzene has Its substituents in a 1, 4 relationships [2].



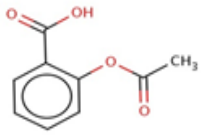
I.2.4. The different families of aromatics

- **The aromatic esters**

Esters are compounds that have the general formula R-COO-R' while the aromatic esters are probably derived significant aromatic acids, because they are very wide spread in nature and are used for very different purposes. **Figure (7)** represents a few examples of aromatic esters [2].



methyl-2-hydroxybenzoate

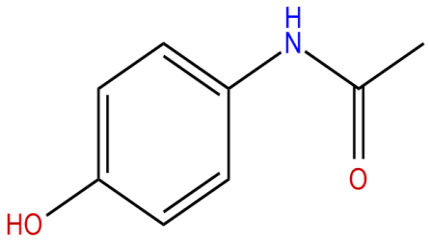


2-(acetyloxy) benzoic acid

Figure I. 7 Some aromatic esters

- **Aromatic Amides**

From derivatives of aromatic carboxylic, acids include, aromatic amides, which correspond to the general R-CON-R formula, and are less susceptible to nucleophile attack. An example of aromatic amide is shown in the following figure [3].



N-(4-hydroxyphenyl) acitamide

I.2.5. Physical and chemicals proprieties

- **Physicals**

Aromatic compounds are generally nonpolar, Liquids or solids with characteristic. They are insoluble in water but are miscible in all proportions with organic solvents such as ethanol, ether etc. They are inflammable and burn with sooty flame, toxic and carcinogenic in character. The boiling points increase when the molecular weight increase, but their melting points do not exhibit regular gradation melting point to depend on molecular symmetry then on molecular weight [3].

- **Chemicals**

Aromatic compounds, originally named because of their fragrant properties, are unsaturated hydrocarbon ring structures that exhibit special properties due to their aromaticity, including an unusual stability. They are often represented as resonance structures containing single and double bonds. However, the bonding is stronger than expected for a conjugated structure, and it is more accurately depicted as delocalized electron density shared between all the atoms in the ring [3].

I.2.6. Used of aromatic compounds

Aromatic compounds play key roles in the biochemistry of all living things. The four aromatic amino acids histidine, phenylalanine, tryptophan, and tyrosine each serve as one of the 20 basic building-blocks of proteins. Further, all 5 nucleotides (adenine, thymine, cytosine, guanine, and uracil) that make up the sequence of the genetic code in DNA and RNA are aromatic purines or pyrimidines. The molecule heme contains an aromatic system with 22 π electrons. Chlorophyll also has a similar aromatic system.

Aromatic compounds are important in industry. Key aromatic hydrocarbons of commercial interest are benzene, toluene, ortho-xylene and Para-xylene. About 35 million tonnes are produced worldwide every year. They are extracted from complex mixtures obtained by the refining of oil or by distillation of coal tar, and are used to produce a range of important chemicals and polymers, including styrene, phenol, aniline, polyester and nylon [2].

Chapter II :
Overview of antibiotics

II. Overview of antibiotics

II.1. History

Antibiotics have revolutionized medical care in the 20th century, but in recent years bugs have been winning the battle against the medical profession. Penicillin was the first antibiotic discovered by Alexander Fleming in 1929, but it was not until the early 1940s that its true potential was acknowledged and large scale fermentation processes were developed for the production of antibiotics. They have been used to treat a wide variety of often dangerous illnesses caused by bacteria. In the early years, new antibiotics were developed faster than bacteria developed resistance to them. But the bugs have fast caught up. In the 1950s and 60s, many new classes of antibiotics were discovered. But in the 1980s and 1990s, scientists have only managed to make improvements within classes. [4]

II.2. Definition of antibiotics

The antibiotics are Substance (such as penicillin) that destroys or inhibits the growth of other microorganisms and is used in the treatment of external or internal infections. While some antibiotics are produced by microorganisms, most are now manufactured synthetically [5].

II.3. types of antibiotics

The great number of diverse antibiotics currently available can be classified in different ways, e.g., by their chemical structure, their microbial origin, or their mode of action. They are also frequently designated by their effective range. Tetracyclines, the most widely used broad-spectrum antibiotics, are effective against both Gram-positive and Gram-negative bacteria, as well as against rickettsia and psittacosis-causing organisms (see Gram's stain). Ciprofloxacin (Cipro) is another broad-spectrum antibiotic, effective in the treatment of mild infections of the urinary tract and sinuses. The medium-spectrum antibiotics bacitracin, the erythromycins, penicillin, and the cephalosporin are effective primarily against Gram-positive bacteria, although the streptomycin group is effective against some Gram-negative and

Gram-positive bacteria. Polymixins are narrow-spectrum antibiotics effective against only a few species of bacteria [5, 6].

II.4. The mode of action of antibiotics

Different antibiotics have different modes of action, owing to the nature of their structure and degree of affinity to certain target sites within bacterial cells [4].

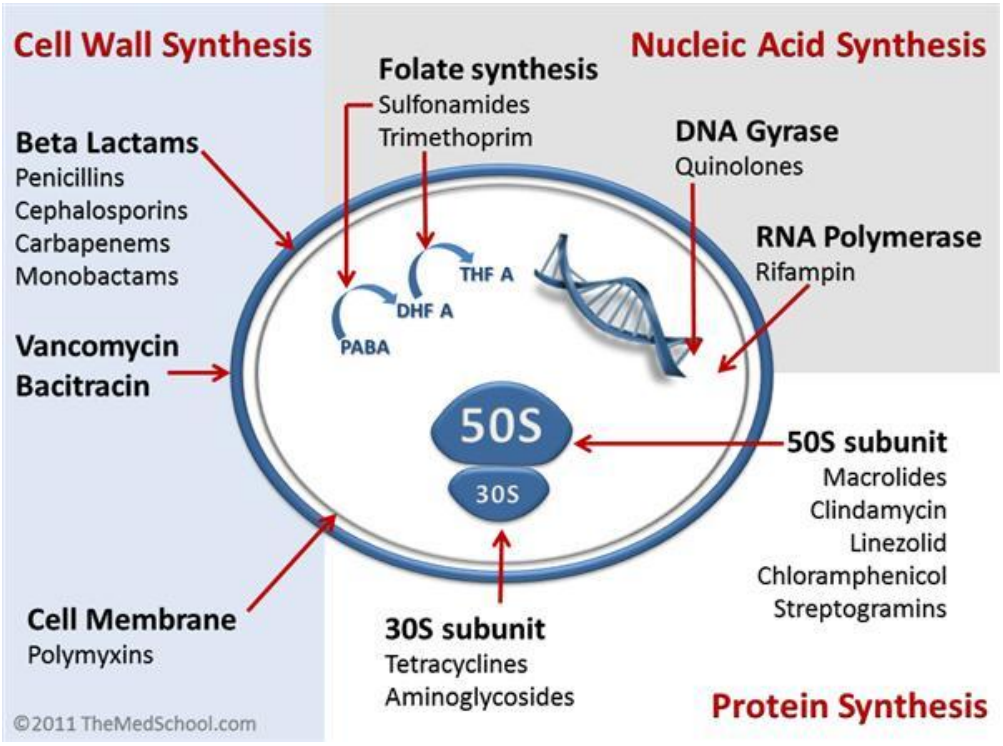


Figure II 1 Antibiotics' mode of action

II.4.1. Inhibitors of cell wall synthesis

While the cells of humans and animals do not have cell walls, this structure is critical for the life and survival of bacterial species. A drug that targets cell walls can therefore selectively kill or inhibit bacterial organisms. Examples: penicllins, cephalosporin, bacitracin and vancomycin [7].

II.4.2. Inhibitors of cell membrane function

Cell membranes are important barriers that segregate and regulate the intra- and extracellular flow of substances. A disruption or damage to this structure could result in

leakage of important solutes essential for the cell's survival. Because this structure is found in both eukaryotic and prokaryotic cells, the action of this class of antibiotic are often poorly selective and can often be toxic for systemic use in the mammalian host. Most clinical usage is therefore limited to topical applications. Examples: polymixin B and colistin [7].

II.4.3. Inhibitors of protein synthesis

Enzymes and cellular structures are primarily made of proteins. Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cells. Several types of antibacterial agents target bacterial protein synthesis by binding to either the 30S or 50S subunits of the intracellular ribosomes. This activity then results in the disruption of the normal cellular metabolism of the bacteria, and consequently leads to the death of the organism or the inhibition of its growth and multiplication. Examples: Aminoglycosides, macrolides, lincosamides, streptogramins, chloramphenicol, tetracyclines [7].

II.4.4. Inhibitors of nucleic acid synthesis

DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in the process of DNA or RNA synthesis, which causes interference of the normal cellular processes which will ultimately compromise bacterial multiplication and survival. Examples: quinolones, metronidazole, and rifampin [7].

II.4.5. Inhibitors of other metabolic processes

Other antibiotics act on selected cellular processes essential for the survival of the bacterial pathogens. For example, both sulfonamides and trimethoprim disrupt the folic acid pathway, which is a necessary step for bacteria to produce precursors important for DNA synthesis. Sulfonamides target and bind to dihydropteroate synthase, trimethophrim inhibit dihydrofolate reductase; both of these enzymes are essential for the production of folic acid, a vitamin synthesized by bacteria, but not humans [7].

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II.5. Classification of antibiotics

- **The first classification is according to the spectrum.**

The spectrum means the number of the organisms affected by the same drug. There are narrow and wide spectrum antibiotics. The wide spectrum antibiotics affect several types of bacteria and fungi and it is usually used where the specific type of the microorganism is unknown. For example, when we are treating an bacterial caused inflammation, we know that we are dealing with a staphylococcus or streptococcus microorganism so the doctor can proceed with the treatment without asking for more lab tests to identify the specific type of the microorganism using the broad spectrum antibiotics but in other cases, where we know the specific type of the microorganism, we can use the narrow spectrum antibiotics that are more effective on specific microorganism but less effective on others [8].

- **The second classification is according to the type of the action of antibiotics.**

It could be bactericidal or bacteriostatic. The bactericidal antibiotics kill the harmful microorganism while the bacteriostatic ones tend to slow down their growth and give the body the chance to use its immune system against the microorganisms. In case of virulent microorganisms or in case of weak immunity, bactericidal antibiotics are preferred because they will omit the problem from its roots but they will affect the normal microorganisms in the body. In mild cases, bacteriostatic antibiotics could be used because of their minor side effects [8,9].

- **The third classification of antibiotics is according to the route of administration of the drug.**

The prevalent route of administration is the oral route but, there are other routes of administration that are more effective in certain cases like injection or topical applications [8].

II.6. Penicillins

II.6.1. Introduction

Penicillin, the first true antibiotic, was discovered in 1928 by Alexander Fleming, Professor of Bacteriology at St. Mary's Hospital in London who scientifically proved its effect and tested it on humans. In the past, scientists such as Louis Pasteur and Ernest Duchesne came across this antibiotic and its anti-bacterial effects. However, they failed to convert it into a medical breakthrough. It was in 1938 when Howard Walter Florey, Ernst Boris Chain and Norman Healthy collaborated with Alexander Fleming and formulated a method to produce penicillin in a large- scale basis. The drug became very popular due to its high activity rate and low toxicity. It became known as a "wonder drug" which cured many different types of diseases. Since then, it has become the drug of choice for the most common bacterial infections [8].

II.6.2. The general structure

Penicillin consists of a Thiazolidine ring (α) attached to a β – lactam ring. The rings constitute the fundamental nucleus of all the penicillin called 6-amino-penicillanic acid (6-APA). β- Lactam ring carries a free amino group to which acid radical can be attached to synthesis newer penicillin [10].

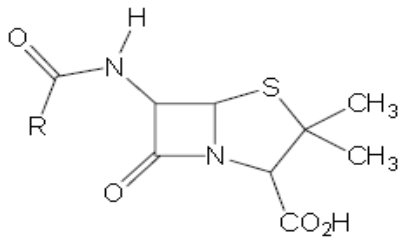


Figure II 2 The general structure of penicillins

II.6.3. Nomenclature

The penicillins are commonly named as penams; a designation in which the sulphur atom is given the top priority.

Using this nomenclature, the penicillins have a prerequisite carboxylic acid group placed at the C-3 position. The west-end substituents are joined to the C-6 Centre and are usually substituted via acylation, thus constituting a variety of C-6 acylamido substituent.

The ß-lactam carbonyl Centre is located at position 7 and the C-2 Centre contains a geminal dimethyl substitution characteristic of penicillins [11].

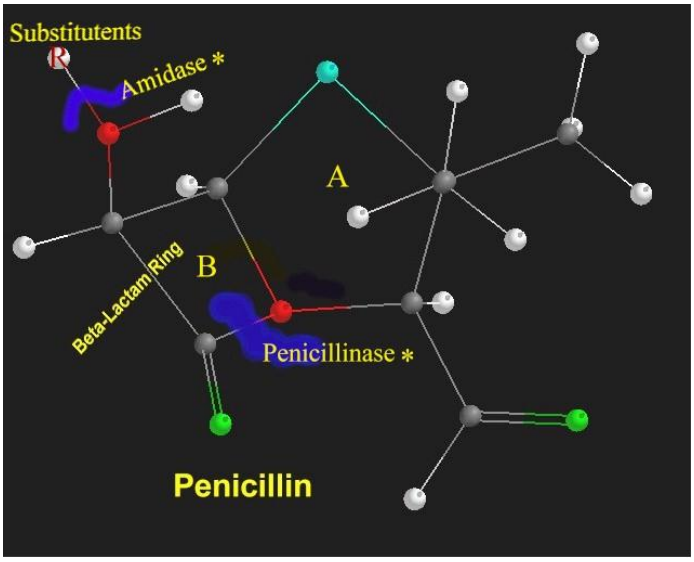


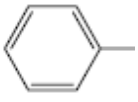
Figure II 3 Structure of 6-aminopenicillanic acid

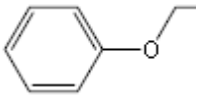
They are called Acid-6 Amino-penicillanic and general terms it is called by putting the name of the root R before the word penicillin [11].

II.6.4. Modes of obtaining penicillins

➢ Mode of obtaining natural penicillins

Among the many natural types of penicillin, differing in the radical R Acylating the amino-6 Penicillanic, only two remain to be used therapeutically:

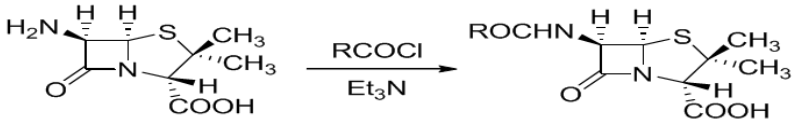
- Penicillin G or Benzyl penicillin R= 

- Penicillin V or Phenoxy Methyl Penicillin R= 

And are always obtained by means of biotechnological processes: cultures of Penicillium [1].

II.6.5. Mode of obtaining semisynthetic penicillins

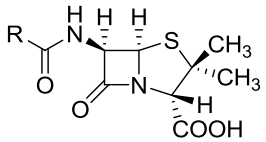
The general mode of obtaining the semi synthetic penicillins is the following, always based on obtaining the amino-6 penicillanic (6-APA), followed by acylation of the substitution on the amino function with chlorides acyl most often in acetone for example, in the presence of trimethylamine to absorb HCl formed [1].

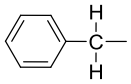
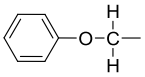
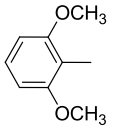
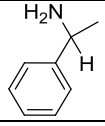


6 acid - aminopenicillanic R- Penicillin

Scheme II 1 How to obtain semi-synthetic penicillins by acylation of 6-APA

Depending on the nature of the substituents of 6-aminopenicillanic acid, there are different classes of compounds **(*table*1):**



penicillins	Nomenclature	the side chain
penicillin G	Benzylpenicillin	
penicillin V	phenoxymethylpenicillin	
Penicillin M	Methicillin	
penicillin A	ampicillin	

II.6.6. The chemical characteristics of penicillins

- **Characteristics returning to the carboxyl group (-COOH)**

Penicillins are organic acids that can soluble in water, but they are soluble in organic solvents, and after using this group, penicillins give:

a. Minerals

They are Alkali metal salts that are soluble in water, and the form is most commonly used in medical care [4].

b. The salts of the amines

Not soluble in water, they have a relatively larger molecular weight, and they also hydrate slowly in organic solvents.

c. Formation of esters

- **Characteristics accruing to β-lactam cycle**: The ease of hydration of the cycle and its openness precise its chemical characteristics. The opening of the β-lactam cycle produces the effect of nucleophilic and electrophonic chemical sensors [1].

- **The accruing characteristics to the Acyl group (R-CO):** Penicillins are characterized from each other by the quality R-CO group bonded to the carbon (6) by the amine function [6].

II.7. Combinations of antibiotics

The use of a combination of the Antibiotics is justified in four instances:

- broaden the spectrum in the case of multiple germs infections
- emergency treatment undiagnosed infection
- prevent the selection of resistant mutants [5].

- **The combination of an antibiotic x and an antibiotic y by agar diffusion method in-vitro study**

  - **Principle**
    - ✓ Two qualitative techniques allow evaluation
    - ✓ Placement Close-up of two discs to the surface of an agar medium, to detect synergies or the sharpest antagonisms
    - ✓ Absorbent papers trips impregnated with antibiotics.
    - ✓ They are placed vertically on the agar medium. After the release of two antibiotics on agar, there are several effects on microbial growth [9, 11].

  - **Synergy**

    The effect of the combination is greater than the sum of the effects produced by each of the antibiotic alone .Cotrimoxazole is an example of synergistic combination of antibiotics (trimethoprim -sulfamethoxazole).

  - **Antagonism**

    The effect of the combination is less than the sum of the effects produced by each of the antibiotic alone.

  - **Indifference**

    Activity of an antibiotic has no influence on the activity of the other.

  - **Addition**

    The effect of the combination is equal to the sum of the effects produced by each of antibiotics taken alone [6].

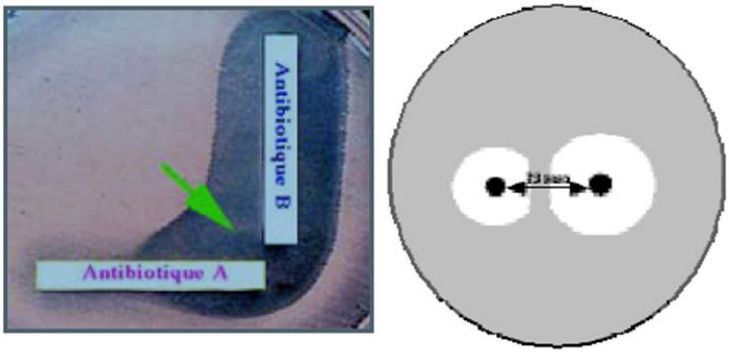


Figure II 4 Proof of synergy

Chapter III :
General bacteriological

III. General bacteriological

III.1. Overview of bacteria

Bacteria are tiny organisms that can be found almost everywhere. They sometimes show their presence-of wound infections, the sunt milk, meat rots, but usually we ignore them because their activities are less obvious and because of their small size.

In 1673, Antoni van Leeuwenhoek (1632-1723) was the first to observe bacteria which he called Animalcules. In addition to the first description of red blood cells and sperm, Dutch Drapery observed bacteria for the first time and described their forms.

It was not until two centuries later that the role of bacteria in fermentation processes and in the transmission of bacteria was discovered and their study began. The most famous scientists of the time were Louis Pasteur and Robert Koch [6, 12].

III.1.1. Bacteria

The bacterium is an independent unicellular small (microorganism). The bacterial cell has a prokaryotic organization differs markedly eukaryotic cells of animals and plants [6].

III.1.2. The structure of the bacteria

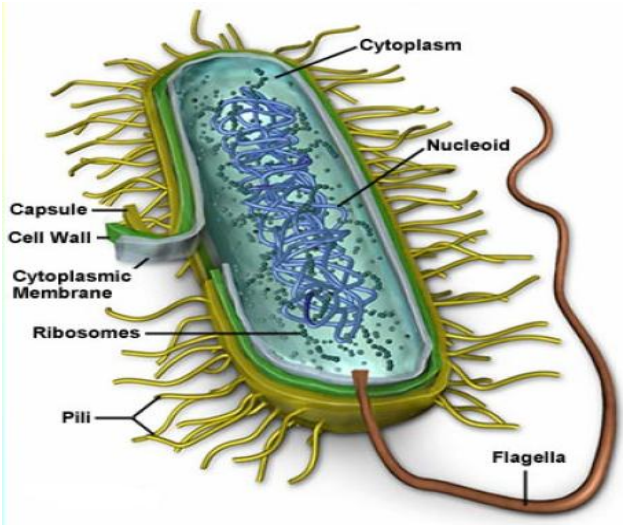


Figure III 1 The structure of the bacteria

In general, a bacteria consists of: [13, 12]

✓ **Cytoplasm:** the bacterial cytoplasm contains ribosomes and many chromosomes DNA is "double-stranded, typically single, and circular".

✓ **Cytoplasmic Membrane:** Surrounding the cytoplasm and is the boundary with the external environment.

✓ **Cell wall**: It gives shape to the bacteria and protects against osmotic lysis. It has components that contribute to pathogenicity.

They protect against toxic substances, it is the site of action of antibiotics. The wall structure varies bacteria and determines their appearance after Gram staining.

After staining there are two types of bacteria: Gram-positive bacteria and gram-negative bacteria.

✓ **The capsule:** Additional layer on the outside of the wall composed of polysaccharides.

The capsule is a well-organized layer while the layer is composed of mucoid substance diffuses unorganized. It Provides resistance to phagocytes, the dryness, repels viruses and detergent, and allows attachment to solid surfaces.

III.2. Appendices

Certain bacteria can move through in a liquid medium to flagella protein in nature. Some bacteria also have pill, they are shorter than flagella rigid components of protein nature. They may be involved in interactions with other bacteria or eukaryotic cells.

III.3. Classification and identification of bacteria

Bacteria are classified by an international nomenclature, they are designated by two Latin words in italics: the first, beginning with a capital letter, refers to the genre, the second

beginning with a lowercase letter characterizes the species (e.g. Staphylococcus aureus). In practice, also common terms such as staph, E. coli are used, etc. Classification bacteria (taxonomy) were originally based on the study of their IR phenotypic and genotypic characteristics [1].

III.4. Definition of resistance

Bacterial resistance to antibiotics is either natural or acquired [13].

III.4.1. Natural resistance

Natural or intrinsic resistance against an antibiotic is present for every character wild strains of the same bacterial species. The natural resistance of bacterial species towards an antibiotic explains the spectrum of activity of an antibiotic.

The natural resistance of bacteria against antibiotics derived from genetic factors of the bacterium. It depends on metabolic characteristics and structure of these species.

It may be due to:

- Impermeability of the bacterial cell wall.
- The secretion of enzyme inactivating the antibiotic.
- The absence of a molecular target for the antibiotic or lack of action site.

III.4.2. Acquired resistance

The acquired resistance for bacterial species to antibiotics is due to a change in the information of the wild-type bacteria. It comes from new genetic information located on the chromosome or on a plasmid R (or a transposon).

III.4.3. Bacteria can result

- A mutation in the genes of the bacterial chromosome.
- From the acquisition of plasmids by transduction or conjugation.
- The acquisition of a transposon.

III.5. Methods for the Study of bacteriostatic [1,13,6].

III.5.1. Definition of bacteriostatic

This method is a technique that allows knowing if the antibiotic only inhibited growth.

III.5.2. Dilution method

- **Liquid milieu**: Preparation of a series of tubes of a range of antibiotic concentrations to be tested (for example 0.5 mg / l, 1, 2, 4, 6, 8 mg / l) and then addition of the same amount of seed. After incubation at 37 ° C for 18 hours to determine the lowest concentration of antibiotic inhibiting bacterial growth visible to the naked eye.

- **Solid milieu:** Incorporation of a given antibiotic in agar concentration, liquid maintained at 42°C then poured into Petri mold. After solidification boxes are seeded with bacteria. After incubation at 37 ° C for 18 hours by determining the lowest concentration giving no visible growth.

III.5.3. Method of distribution

Dissemination methods or standards susceptibility are mostly used by diagnostic laboratories. Blotting paper disks impregnated antibiotics tested at a concentration deposited on the surface of an agar medium previously inoculated with a pure culture of the strain to study. After overnight incubation at 37 ° C; the discs are surrounded by circular inhibition zones corresponding to a lack of culture. Each zone can be measured by various means in mm, and then it will be possible to calculate the MIC of the antibiotic for the strain examined by plotting the diameter of a standard curve.

III.5.4. E-test

A gradient of antibiotic concentrations is obtained in a plastic strip. After the deposition of the strip on the surface of a Petri mold, seeded with the suspension of the bacterium to be tested, and after overnight incubation at 37 °C, the value of the diameter of inhibition is read at the intersection.

III.6. Reading the susceptibility

For each antibiotic we measure the inhibition diameter by mm and deduce the sensitivity or the resistance, we define two critical concentrations of antibiotic:

- **CMI**=the lowest concentration of antibiotic for which there is no visible growth of the bacterial strain studied, the culture conditions are standard.
- **CMB**= the lowest concentration of antibiotic leaving less than 0.01% of survivors of an initial inoculum [1, 13, 6].

Chapter IV:
Methods and Materials

IV. Methods and Materials

IV.1. Introduction

The disc-plate method of determining which of a wide variety of antibiotics is antagonistic to an organism is a rapid, accurate, and inexpensive diagnostic tool. This procedure gives the physicians information regarding the effective microbial drugs to use in the chemotherapy of infectious diseases. This is important because treating an infection with an inappropriate antibiotic will not only not help the situation, it may make things worse by destroying competitors of the infectious agent. In addition, if there are individuals within the bacterial population that are resistant to the antibiotic being used, these organisms will be selected for, through elimination of organisms that are sensitive. If organisms are only marginally sensitive, it may take higher concentrations or longer durations to completely destroy the population. It is for this last reason that it is important to complete an antibiotic regimen once started, in order to avoid selecting the antibiotic resistance.

In the disc-plate method, a number of small discs impregnated with an antibiotic of known concentration are placed on the surface of an agar plate that has been inoculated with the organism to be tested. After proper incubation, the plate is observed and zones of inhibition are measured to determine the susceptibility of the test organism to particular antibiotics. Based on the size of the zone of inhibition the particular microbial agent will be designated as resistant, intermediate, or sensitive to the test antibiotic. The chart accompanying this exercise lists the appropriate zone sizes for each antibiotic tested.

IV.2. Objective

The main objective of our study is to test the biological effect of salicylaldehyde with 6-APA nucleus acid.

IV.3. Work Methodology

The methodology adopted for this study is based on:

- The study of the susceptibility of bacteria (E. coli, pseudomonas, staphylococcus aureus) in Salicylaldehyde and 6-APA acid nucleus.

- A comparison between the results of the effect of each product on the test strains and the results of the association of two products effect.

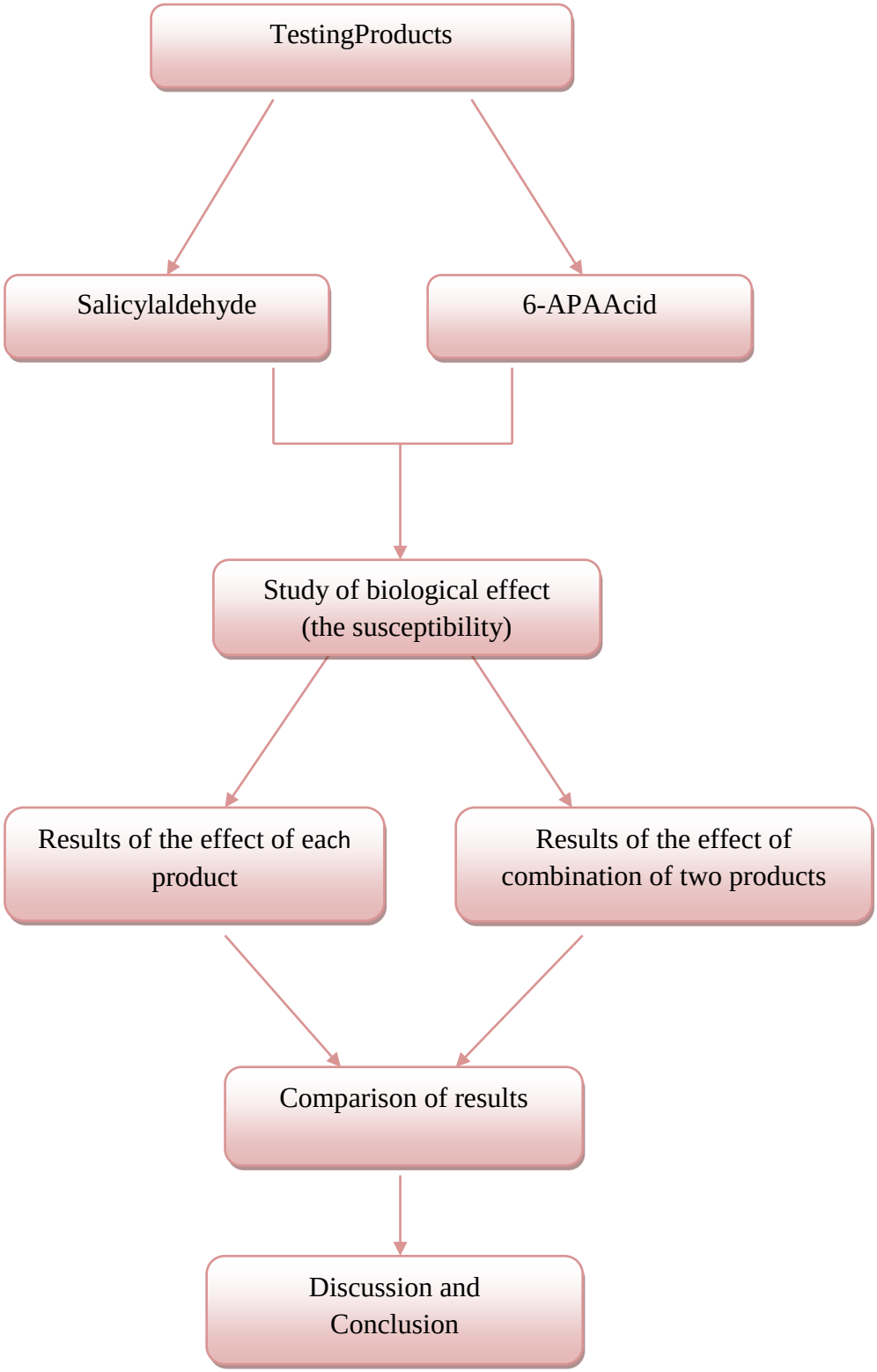


Figure IV1 plan of the Works Methodology

IV.4. Experimental conditions

- **Reagents and solvents**

The list of these products is shown in the following table:

Table IV. 1 Reagents and Solvents

Reagents and solvents	**purity%**	**Origin**
Salicylaldehyde	/	Fisher scientific
Chloroform	96	Prolap
6-APA	/	SAIDAL
Sodium hydroxide	99.5	Biochemchernopharma

- Nucleus of the 6-APA acid is identified by the methods of physico-chemical analysis according to their IR and NMR spectra. This product is developed at the level of SAIDAL laboratory.

- **Materials*:***

- Graduated cylinder
- Pipet 1ml and 5ml
- Pasteur pipets
- Beaker
- Filter paper
- Magnetic Bar
- Thermometer
- Disk filter paper test tube
- Distilled water
- Water physiological
- Oven-magnetic agitator
- Balance (Scout Pra).

IV.5. Used bacteria

(E. coli- Pseudomonas- staphylococcus aureus)

IV.6. Bacteriological technique

- We studied the sensitivity of the test strains towards the two products (nucleus 6-APA acid and Salicylaldehyde) by the diffusion method (standard sensitivity). Then, the combination volume to volume of 6-APA acid with Salicylaldehyde to study the synergistic effect.
- **Concentrations used in the tested products:** The tested products are soluble in distilled water so it was used as solvent water and then we tested the toxicity of the solvent (pre-test).We made an interval of dilution 20000-200µg/ml for each product, which we prepared a stock solution 20mg/ l of 6-APA acid (0.1g added to 4, 62.10^{-4} Of NaOH in 5ml of water), and another Salicylaldehyde 20mg/l (0.1g in 5ml of chloroform), in order to prepare other concentrations that are stated in the tables below.

Table IV.2 Variety of dilution of the 6-APA acid

Stock solution ($\mu g.ml^{-1}$)	Initial solution (ml) brought from the stock solution	The added Distilled water (ml)	The Aim ($\mu g.ml^{-1}$)
20000	1.5	1.5	10000
(5)ml	0 .75	2.25	5000
	0.3	2.7	2000
	0.03	2.97	200

Table IV.3 Variety of dilution of the Salicylaldehyde

Stock solution (µg.ml-1)	Initial solution (ml) brought from the stock solution	The added Chloroform (ml)	The Aim ($\mu g.ml^{-}$)
20000	1.5	1.5	10000
(5)ml	0 .75	2.25	5000
	0.3	2.7	2000
	0.03	2.97	200

- **Preparation of paper discs:** To prepare disks 5mm diameter filter paper No.3 is used, and then placed in a test tube sterilized at a temperature of 130 ° C for 45 minutes.
- **Preparation of culture midst:** After the dissolution of Mueller Hinton, poured into Petri molds and the environment until solid is allowed, and ran an oven for 15 minutes to dehumidify.

- **Preparation of the microbial suspension:** Rubbed whenever using Pasteur pipet, three bacterial strains and deposited in test tubes contain a nutrient broth (10 ml), stirring well. The suspension was dispersed in the petri mold, past three times on the same area to ensure that they are fully covered, then aspirate excess thoroughly, and dry the molds in the oven at 37 °C for 15minutes.

- **Preparation of inocula:** The method used for the preparation is the method of VINCENT (JACOBETAL, 1979) [12], we prepare 9 test tubes for each compound which contains dilutions we have already prepared. The filter paper discs of 5 mm diameter are immersed in the tubes and are impregnated with a small amount of our products, then with the help of a plier, after that we leave them on the surface of the petri molds beforehand seeded by microbial Suspension.

- **Test of synergy:** The concentrations of the two products, which show an awareness of the test strain, are associated. Then, we study the sensitivity of the strains by the same previous method.

Chapter V :
Results and Discussion

V. Results and Discussion

V.1. Preparation of 6-aminopenicillanic acid

6-aminopenicillanic acid was prepared in a laboratory SAIDAL and its structure was confirmed by spectroscopic methods such as ^{1}HNMR, IR. The^{1}H NMR spectrum shows five distinct signals at1.47 ppm (s,3H,CH_3), 1.56 ppm (s,3H,CH_3), 4.14 ppm (s, 1H, CH) 4.55 ppm (d, 1H, CH) and 5.39 ppm(d,1H,CH).

The IR spectrum showed two bands Characteristics; at 3500 Cm^{-1} assigned to the stretching vibration of the N-H bond of the primary amine, and1772.5 Cm^{-1}attributed to the stretching vibration of the C=O of the acid, other bands are indicated in the following table.

Table V 1 IR bands of 6- aminopenicillanic acid

Frequency Cm^{-1}	Observation
2983.7 Cm^{-1}	stretching vibration of C-H of CH_3
1624.0 Cm^{-1}	stretching vibration of C=O of lactam
1338.5 Cm^{-1}	stretching vibration of C-O of acid

V.2. Results of bacteriological tests

V.2.1. Preliminary Test

Table V 2 Preliminary test with distilled water

Testing Strain	Seeding without distilled water	Seeding with distilled water
Escherichia coli	Growth	Growth
Staphylococcus aureus	Growth	Growth
pseudomonas	Growth	Growth

Table V 3 Preliminary test with NaOH

Testing Strain	Seeding without NaOH	Seeding with NaOH
Escherichia coli	Growth	Growth
Staphylococcus aureus	Growth	Growth
pseudomonas	Growth	Growth

Table V 4 Preliminary test with chloroform

Testing Strain	Seeding without Chloroform	Seeding with Chloroform
Escherichia coli	Growth	Growth
Staphylococcus aureus	Growth	Growth
pseudomonas	Growth	Growth

According to the results on the tables, we see the growth of bacteria which means that the solutions of distilled water, the NaOH, and chloroform have no influence on bacteria.

V.2.2. Results of the determination of the diameters of inhibition of each strain by the method of diffusion:

- **Test on Escherichia coli**

Table V 5 The effect of compounds on the Escherichia coli

	concentration µg/ml / compounds	20000	10000	5000	2000	200	Category of the strain
Inhibition diameter(mm)	6-APA	-	-	-	-	-	R
	Salycalidihyde	-	-	-	-	-	R

(+): **sensitive to the compound** **(-): resistance**

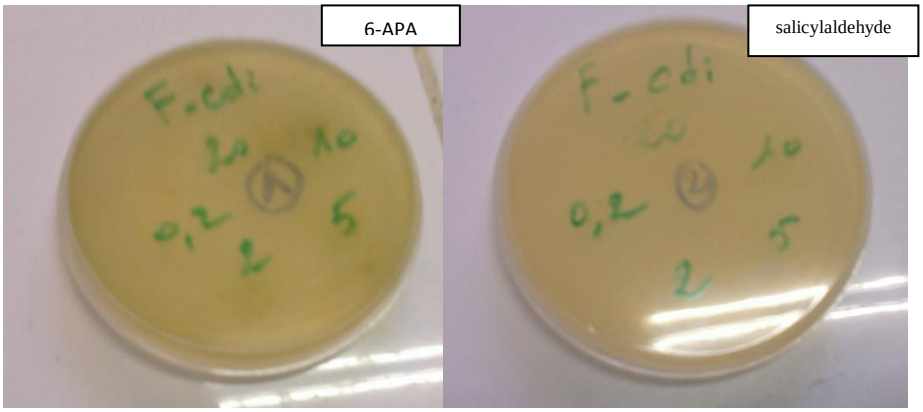


Figure V 1 The effect of salicylaldehyde and acid 6-APA on Escherichia coli

- **Test on Staphylococcus aureus**

Table V 6 The effect of compounds on the Staphylococcus aureus

	Concentration µg/ml / compounds	20000	10000	5000	2000	200	Category of the strain
Inhibition diameter(mm)	6-APA	-	-	-	-	-	R
	Salycalidihyde	-	-	-	-	-	R

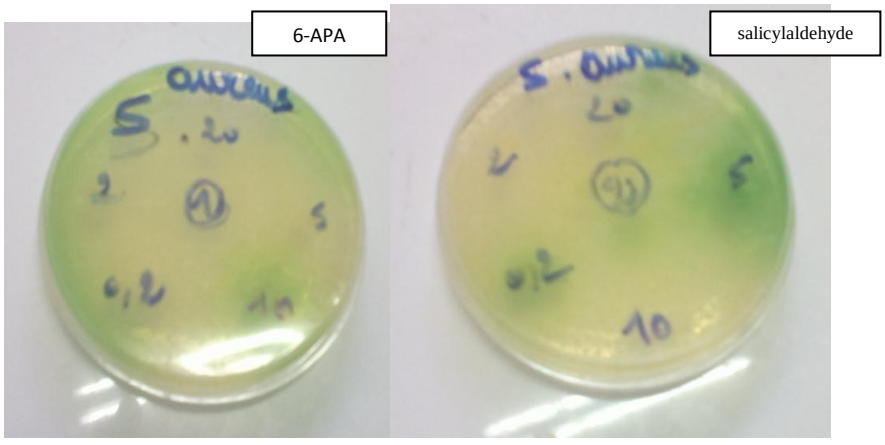


Figure V 2 The effect of salicylaldehyde and acid 6-APA on Staphylococcus aureus

- **Test on pseudomonas**

Table V 7 The effect of compounds on the pseudomonas

	concentration µg/ml / compounds	20000	10000	5000	2000	200	Category of the strain
Inhibition diameter(mm)	6-APA	-	-	-	-	-	R
	Salycalidihyde	-	-	-	-	-	R

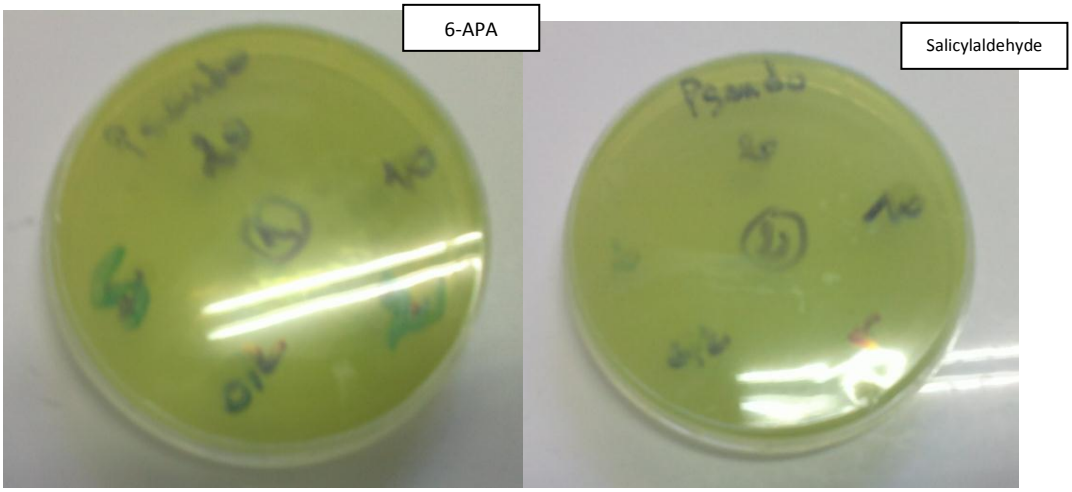


Figure V 3 The effect of salicylaldehyde and acid 6-APA on pseudomonas

According to The results of the tables (V-8, V-9, V-10), and the pictures shown we can see that compounds 6-APA and salicylaldehyde have no effect on the three test strains throughout the concentration range.

V.2.3. Test of synergy

The results of the determination of the inhibition diameters of the three types tested in the case of the combination of the two products (salicylaldehyde andacid6-APA), volume to volume strains are presented in the following table:

Table V8 The test result of the synergy

Concentration of the combination **µg/ml** Salicylaldehyde+ acid 6-APA	Inhibition diameter **(mm)**
2000	_
10000	_
5000	_
2000	_
200	_

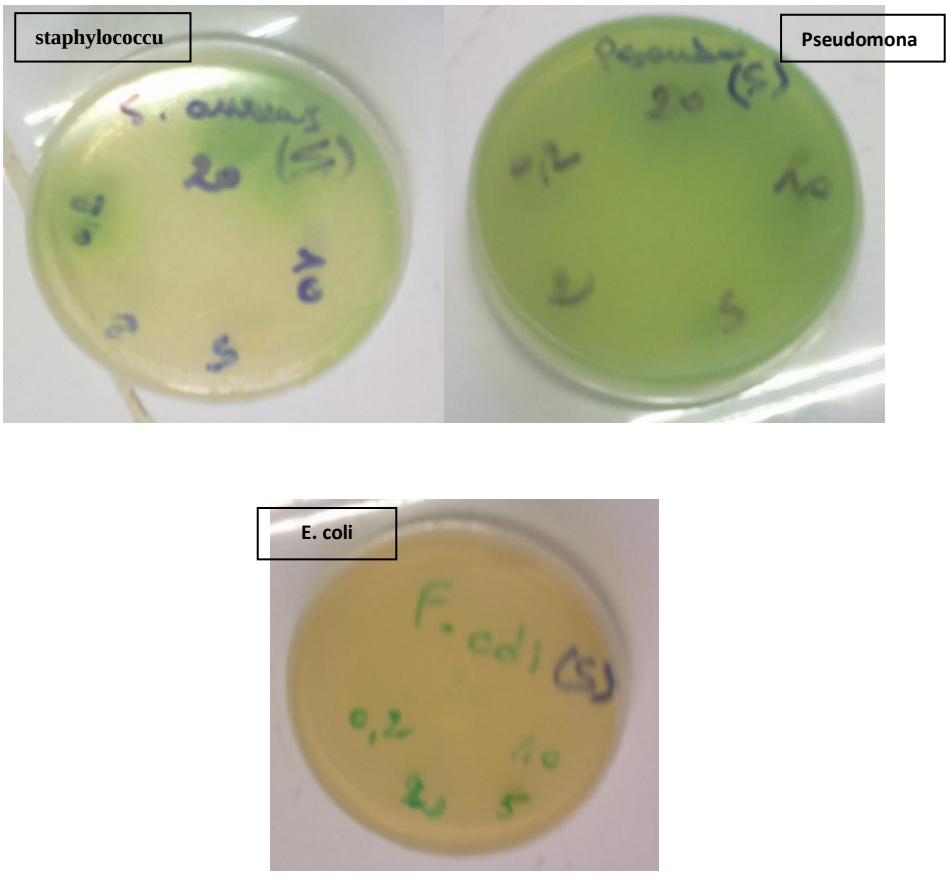


Figure V4 The effect of the association of salicylaldehyde 6-APA onthree types of strains

According to the table above and displayed photos, we observe the resistance of all types of tested strains with the association of the two products. So, the two products do not result a synergistic effect but an effect of indifference.

V.2.4. Conclusion

The 6-aminopenicillanic acid which is the nucleus base of penicillins on the one hand and Salicylaldehyde which was one of the first antipyretic (fever medicine fighter) on the other hand, these products which have no effect on stem bacteria are tested in the field of mergers [20000-200] µg / ml. Even, the sensitivity test which goes by combining two products was not effective. There is not a synergistic effect but an effect of indifference.

General Conclusion

General Conclusion

After having done this research paper we have learnt many things such as: How to work with the laboratory tools, also the microbiological testing, and some techniques of solutions preparations.

Antibiotics are natural substances of biological origin produced by a living organism, a chemical produced by synthetic or semi-synthetic substance produced by chemical modification of a molecule of natural base.

Tests antibacterial activities announced that salicylaldehyde compounds and 6-APA acid had no effect on the three types of bacteria tested, even in the test of association; it cannot be a synergistic effect but an effect of indifference.

To achieve the desired results (sensitivity of bacterial strains to these products) is proposed:

- Increased dose products (increased dilution range).
- Make acylation of 6-APA on its amino function using acyl chlorides.

Résumé :

L'acide 6-amino pénicillinique a été préparé au Laboratoire de SAIDAL et sa structure a été confirmée par l'infrarouge à transformer de fourrier et RMN ^{1}H.

Le produit préparé a été testé avec le Salicylaldehyde sur les bactéries telles que ; Escherichia Coli, Staphylococcus aureus et Pseudomonas, puis l'effet synergique a été déterminé.

Mots clés : acide 6-amino pénicillinique, effet synergique, Salicylaldehyde, bactéries.

Abstract:

The 6-amino penicillanic acid was prepared in SAIDAL Laboratory and characterized by the FITR and RMN ^{1}H.

The product prepared was tested with salicylaldehyde on some bacteria such as; Escherichia coli, Staphylococcus aureus and Pseudomonas, and then the synergistic effect were determined.

Keywords: 6-amino penicillanic acid, salicylaldehyde, bacteria, synergic effect.

ملخص :

تم تحضير حمض 6-امينو بيني سيلانيك في مخبر صيدال ، و تم التأكد من صيغته بواسطة التحاليل المطيافية منها الاشعة تحت الحمراء IR و الرنين النووي المغناطيسي $RMN\ ^{1}H$.

المركب المحضر ثم اختباره مع ساليسيل الدهيد على البكتيريا التالية :البكتيريا القولونية المكورات العنقودية و البسودوموناس و تم تحديد التأثير البيولوجي.

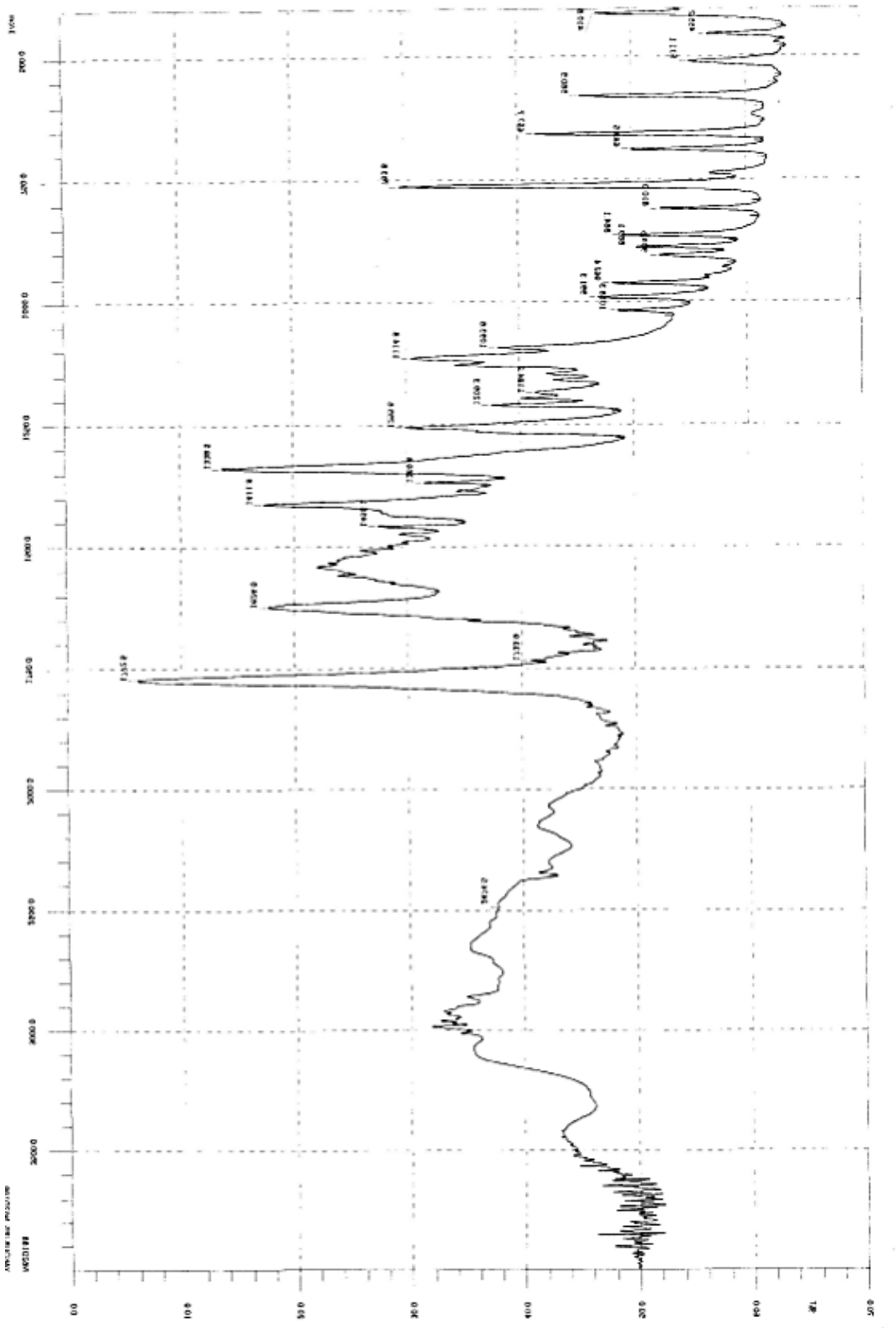
الكلمات الدالة :

حمض 6-امينو بينيسيلانيك، ساليسيل الدهيد، البكتيريا ، تأثير بيولوجي.

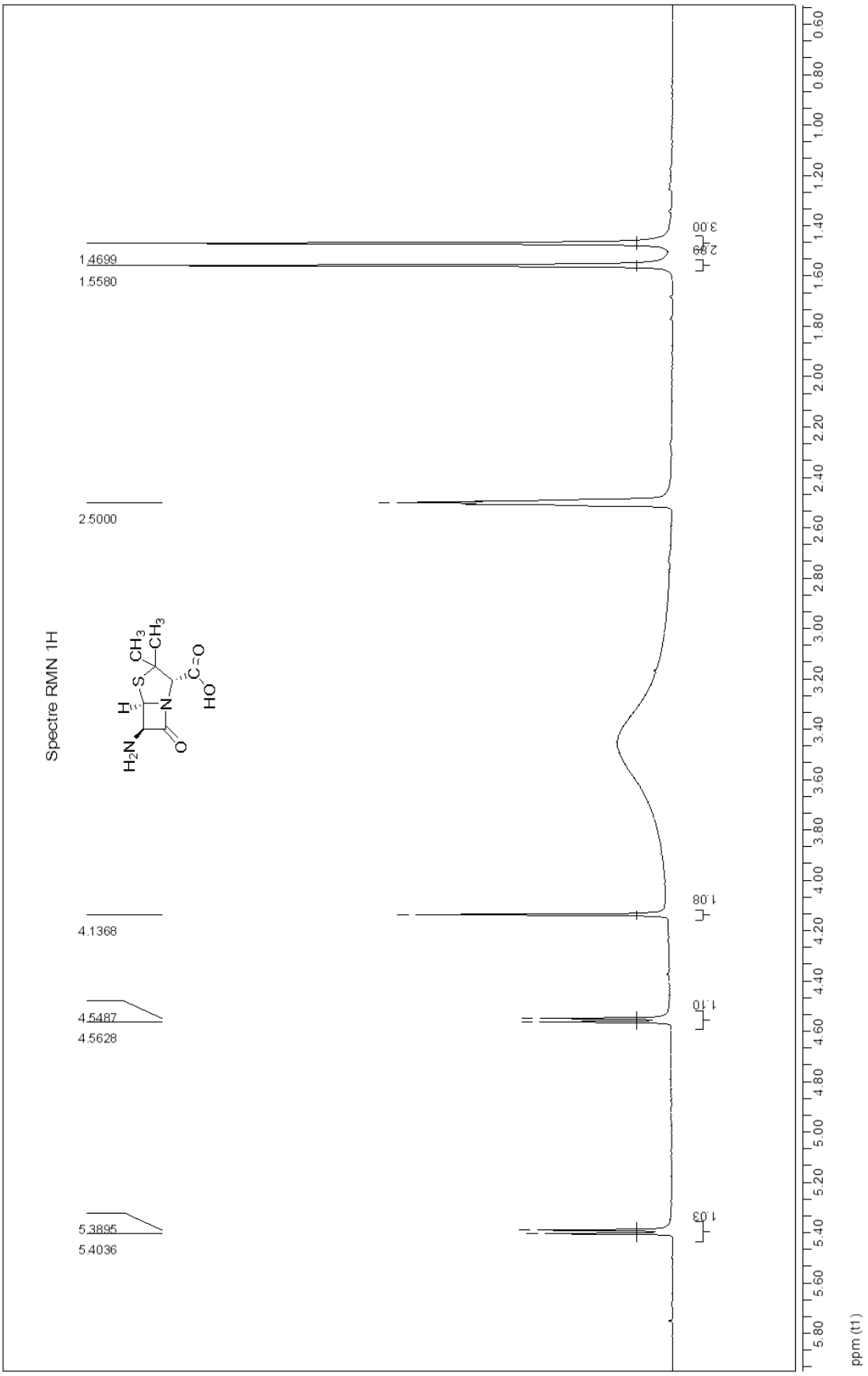
APPENDEX

APPENDEX

IR SPECTRUM OF6-APA



NMR[1]H SPECTRUM OF6-APA



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