

# Preparation, Characterization, Antibacterial Study, Toxicity Study of New Phenylene diamine- Formazan Derivatives

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## Abstract

Formazans are known to be characterized by containing very effective functional groups that have an effect on the possession of any compound with a pharmaceutical and biological characteristic as antimicrobials for many microbes, as these compounds have proven high resistance to the spread of cancerous tumors and the spread of bacteria and fungi. Series of formazan derivatives were synthesized in this study from meta-phenylene diamine via imination reaction then coupling reaction to yield new five derivatives. The formatted formazan derivatives have been investigated through various instrumental -spectral techniques represented by (I.R- Infrared spectra ,H. NMR -Proton magnetic resonance , Mass -Spectra )– as a spectrophotometric measurements ,then bio- studying via various bio- experiments in this field like cytotoxicity, resistance of anti-bacteria.

**Keywords:** pyrimidine, formazan, thiazole, infection, contamination, toxicity, anti-bacteria, phenylene diamine, cancer, tumor.

## Introduction

Cancerous tumors are considered one of the common and widespread diseases in the current century due to the spread of their causes of radiation, wars and their radioactive residues. Therefore, the need to search for antibodies that inhibit the growth of cancer cells and reduce their spread with the least toxicity was necessitated, Therefore, many biological and chemical researchers resorted to finding alternative solutions as treatments and inhibitors for cancer cells, including linking some medical drugs and medicines to some functional groups that affect the growth and reproduction of cancerous tumors.

Formazan is one of the important compounds as a biologically applications<sup>(1-5)</sup> with its many biological treatments<sup>(6-13)</sup>, especially in the field of fungi<sup>(14-16)</sup>, bacteria and malaria treatments<sup>(17-20)</sup>, in addition to

many literatures<sup>(21-28)</sup> in this field that has demonstrated its therapeutic importance in malignant tumors. Several studies<sup>(29-30)</sup> have been conducted to evaluate the role of formazans and their derivatives as reducing the growth and spread of cancerous tumors in the body<sup>(31-37)</sup>,. Formazan compounds are characterized by the diversity of their applications<sup>(38-42)</sup>, studies have shown that formazan works with most of the transitional elements<sup>(43-45)</sup>, which gave vital activity when injected into laboratory animals to test their ability to inhibit the spread of tumors in the body and reduce toxicity, as well as represent dyes tissues<sup>(46-50)</sup> and other applications<sup>(51-59)</sup>.

## Experimental Part :

Formazan derivatives created via numerous chemical processes, checked and reconnoitered by numerous techniques signified in : FT-IR spectra (system 8300 -Shimadzu) -range (400-4000) cm<sup>-1</sup> in KBr discs .,I.H. NMR–Spectra in DMSO–solvent, then Mass -Spectra., also bacterial resistance studies ,in addition to toxicity studies to formazan derivatives.

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**Preparation of Formazan derivatives<sup>(19-22)</sup>:**

**Preparation of Compound {1}:**

m-Phenylene diamine(0.01 mole)reacted by condensation process with (0.02 mole) from vaniline with drops of acid(acetic. glaci.) ,the resulting precipitation dried then re crystallized, by flowing procedures<sup>(19-22)</sup> to yield compound { 1 }.

**Preparation of Compound {2}:**

Compound [1] (0.01 mole)reacted with (0.02 mole) from thiazole diazo salt in basic medium, the resulting precipitation dried then re crystallized, by flowing procedures<sup>(19-22)</sup>, the resulting represents (formazan compounds) compound {2}.

**Preparation of Compound {3}:**

Compound [1] (0.01 mole)reacted with (0.02 mole) from 4-acetothiophene diazo salt in basic medium,

the resulting precipitation dried then re crystallized, by flowing procedures<sup>(19-22)</sup>, the resulting represents (formazan compounds) compound {3}.

**Preparation of Compound {4}:**

Compound [1] (0.01 mole)reacted with (0.02 mole) from pyrimidine diazo salt in basic medium, the resulting precipitation dried then re crystallized, by flowing procedures<sup>(19-22)</sup>, the resulting represents (formazan compounds) compound {4}.

**Preparation of Compound {5}:**

Compound [1] (0.01 mole)reacted with (0.02 mole) from benzothiazole-diazo salt in basic medium, the resulting precipitation dried then re crystallized, by flowing procedures<sup>(19-22)</sup>, the resulting represents (formazan compounds) compound {5}.

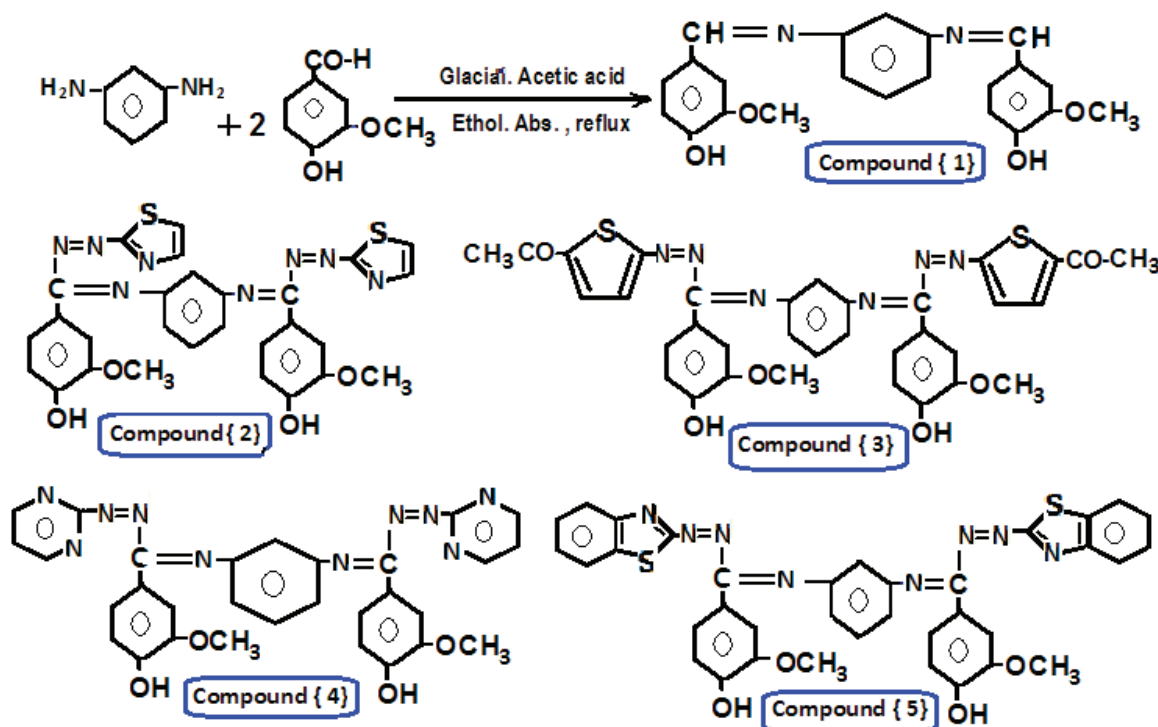
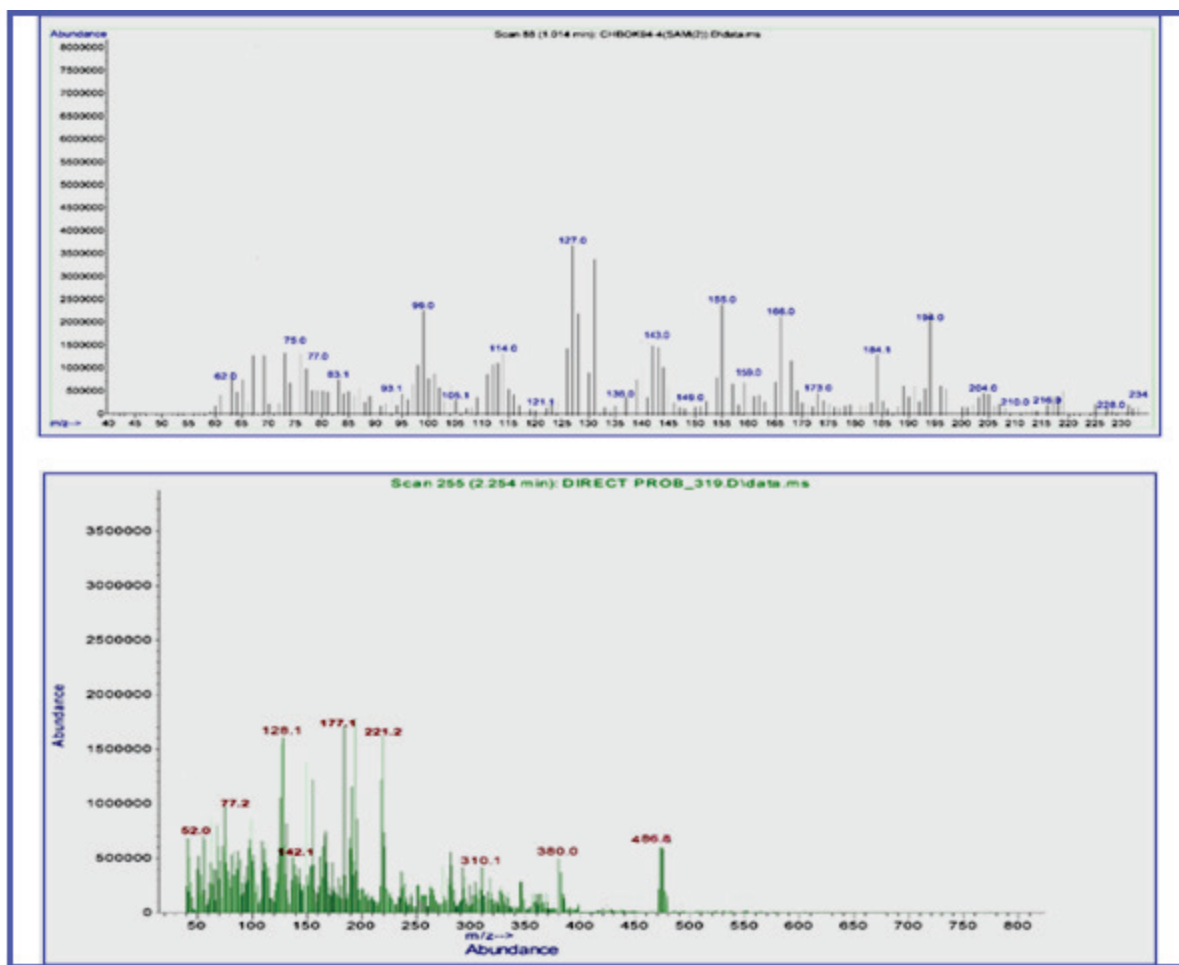


Fig.(1): Preparation of Compounds { 1 - 5 }





Fig(2): Mass –Spectrum of compound {2} , Compound {3}

#### Assay of Cytotoxicity<sup>(30, 34)</sup>

Some of Formazan compounds were screened to evaluate their cytotoxicity against Breast Cancer (AMJ13) and Hela Cancer cells shows that the formatted formazan derivatives gave high data as a results of efficiency of prepared compounds according to studies<sup>(30, 34)</sup>. Compound {5} which included thiazole ring in its structure showed 80% inhibition with an (IC<sub>50</sub> = 1 µg/ml) against (AMJ13) Breast cell and moiety showed 74 % inhibition with an (IC<sub>50</sub> = 1 µg/ml) against Hela cell but it gave on Embryonic cell moiety 38% inhibition with an

(IC<sub>50</sub>= 1 µg/ml) ., While compound {2} appeared 50% inhibition with an (IC<sub>50</sub> = 1 µg/ml) against (AMJ13) Breast cancer and moiety showed 74% inhibition with an (IC<sub>50</sub>=1 µg/ml) against Hela cell while it active on Embryonic cell moiety showed 62% inhibition with an (IC<sub>50</sub>= 1 µg/ml) .

In the present screening study, it was found that increasing in the concentration leads to increasing in the percentage of inhibition .

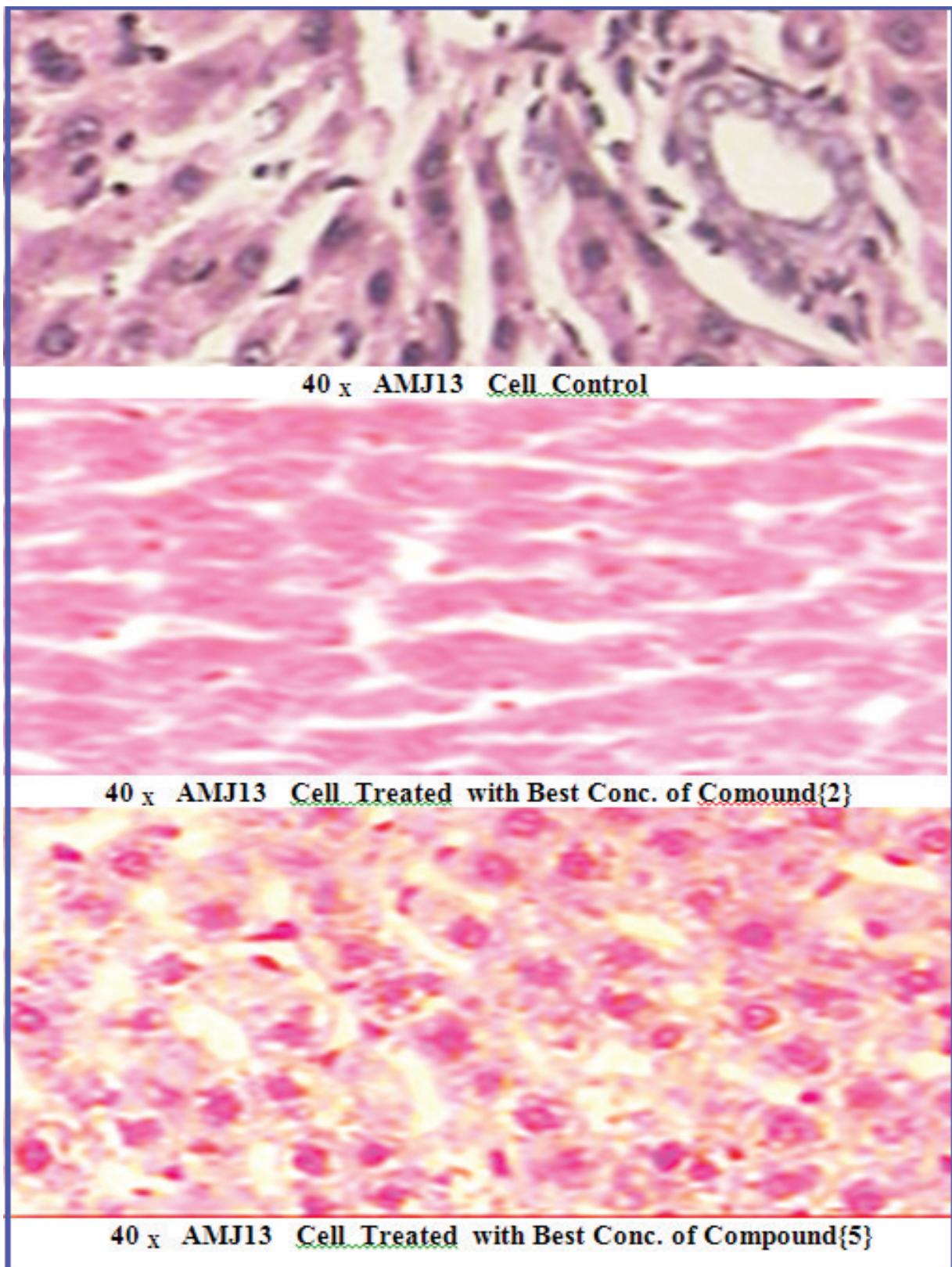
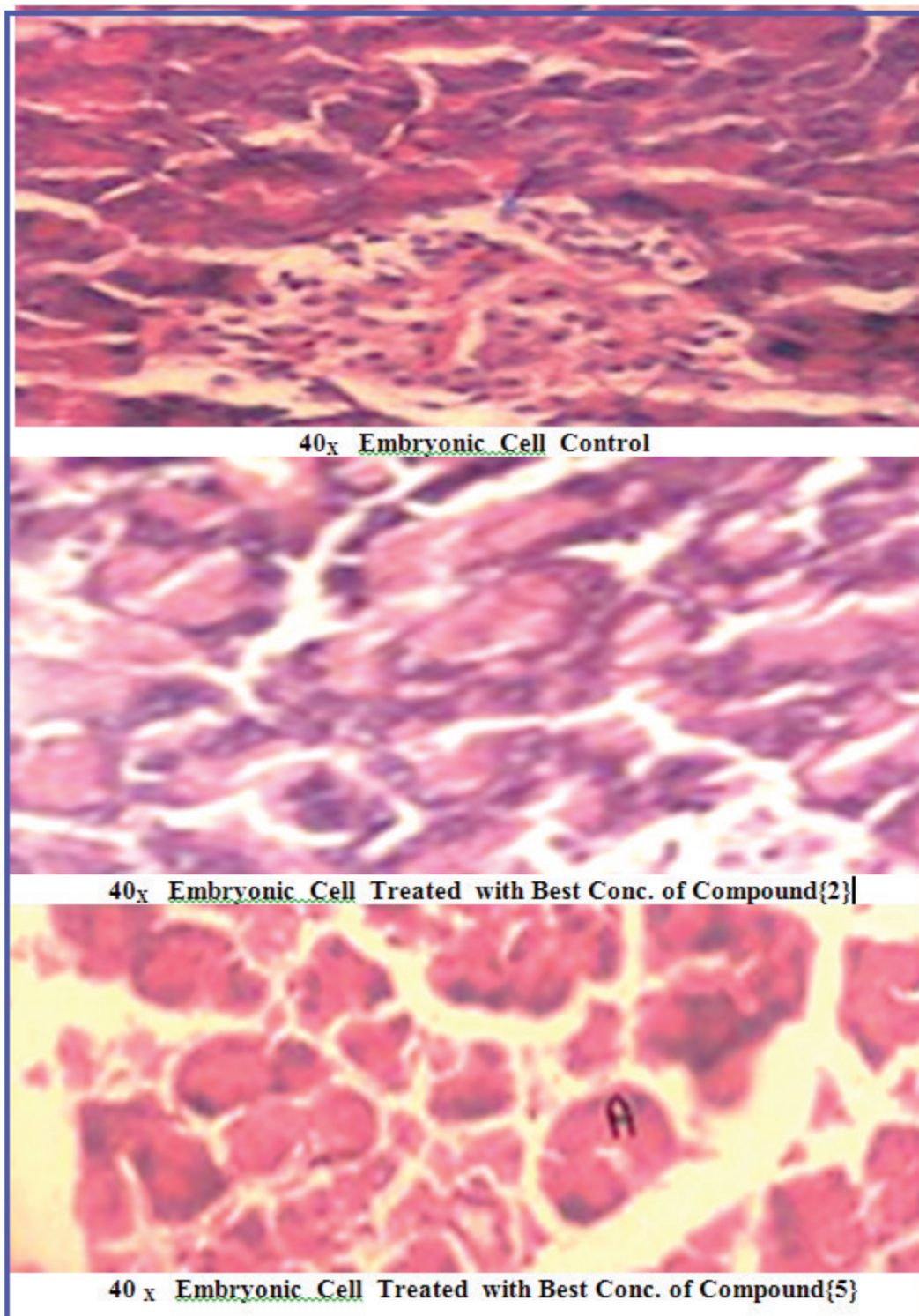


Fig. (4): 40 x AMJ13 Cell Control and Treated of Compounds {2 , 5}



**Fig. (5): 40<sub>x</sub> Embryonic Cell Control and treated of Compounds {2, 5}**

**Selection of Bacteria for Antibacterial Assay<sup>(56-58)</sup> :**

Three types of bacteria were selected for evolution screening towards antibacterial assay represented by (*Salmonella. Typhi* , *P. aeruginosa* , *Streptococcus. Faecalis*) via several concentration of compounds the selected bacteria in Figure . ( 6) :

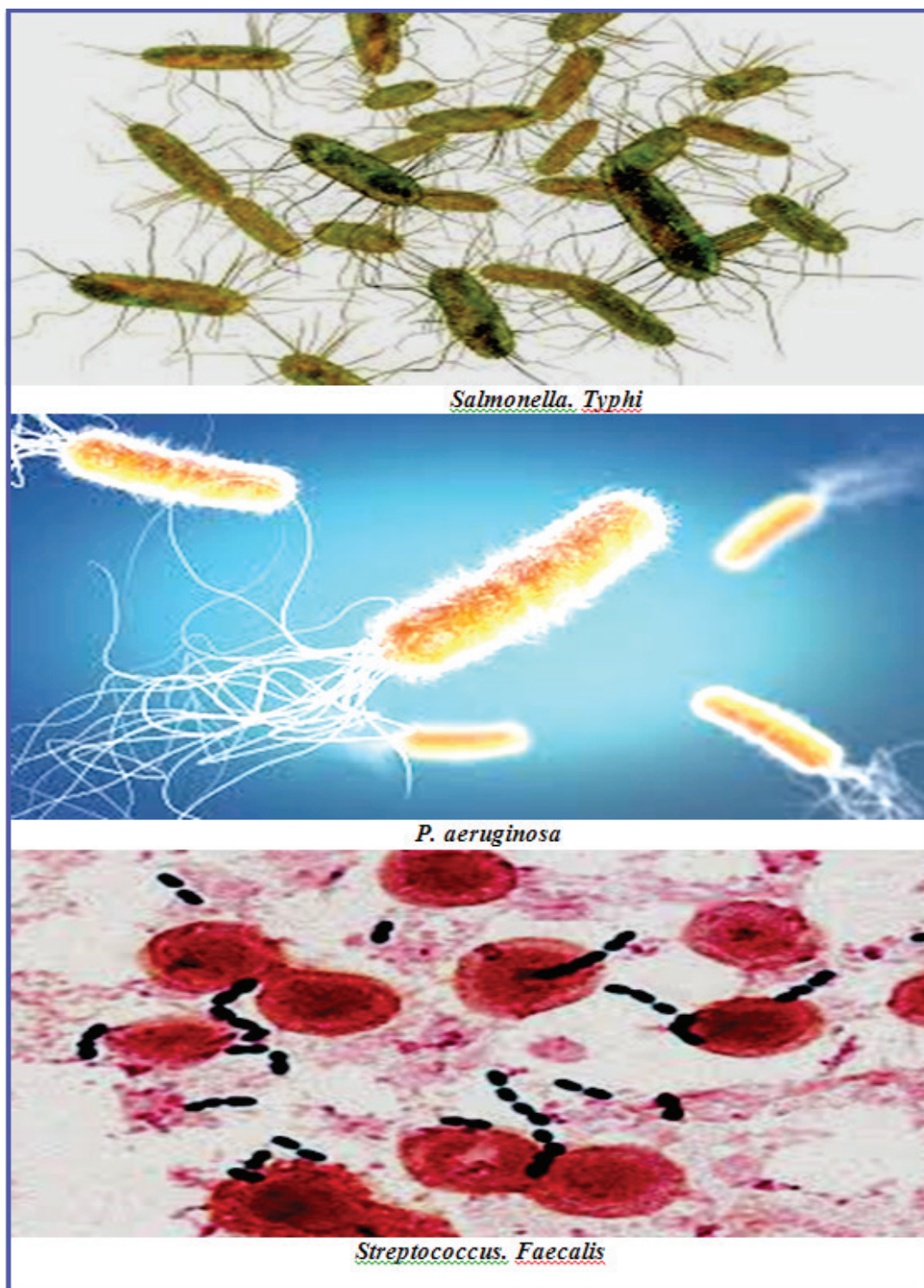


Fig. (6): The selected Bacteria

**Antibacterial Tests<sup>(56-58)</sup> :**

Screening of bio- test for the created formazan compounds have been performed for their antibacterial assay via agar by the flowing numerous procedures<sup>(65, 66)</sup>. The investigation of microbial inhibition carried out at

(three concs) (20, 30 , 40 micro gram) concentrations in best solvent (DMSO) with bacteria :(*Salmonella. Typhi* , *P. aeruginosa* , *Streptococcus. Faecalis* ). The selected types of bacteria incubated for (24 hr) at (37°C).

The test of compounds on three types of bacteria gave high data with compounds {5 and 2} more than other compounds due to ( Sulfur and nitrogen )-atoms in same compounds that participates in inhibition of bacteria., all results in Table (1):

**Table.1: Inhibition test of formazan compounds in Conc. (30 micro gram)**

Cephalexin Derivatives	Salmonella. typhi	Streptococcus. faecalis	P. aeruginosa
Compound {1 }	+	+	+
Compound {2 }	+++	+++	+++
Compound {3 }	++	+++	++
Compound {4 }	++	++	++
Compound {5 }	+++	+++	+++

(+) : inhibition (4-8) mm

(++) : inhibition (9-13) mm

(+++): inhibition (14-18) mm

### Conclusions

The test of compounds on three types of bacteria gave high data with compounds {5 and 2} more than other compounds due to ( Sulfur and nitrogen )-atoms in same compounds that participates in inhibition of bacteria.

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**Ethical Clearance:** Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

**Conflict of Interest :** The authors declare that there is no conflict of interest.

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