UNIVERSITY OF BASRA
COLLEGE OF PHARMACY

EXAMINATION OF ANTIBACTERIAL ACTIVITY OF *SCHIFF BASES* OF SELECTED *NON-ANTIBIOTIC PRODUCTS ACYCLOVIR*

باشراف:
الدكتورة هدى صالح

المرحلة الخامسة

سارة فرحان علي رياض كاظم

ABSTRACT

- Introduction about acyclovir tablets

- Schiff's bases are excellent ligands which are synthesized from the condensation of primary amines (acyclovir drug) with carbonyl groups (aldehydes).

CONCLUSION

Some Schiff's bases synthesis in an ethanolic solution of drug, aldehydes and glacial acetic acid as a catalyst was followed in the synthesis of substituted acyclovir drug compounds. The synthesis compounds were characterize by confirmed by various spectra technique like FTIR addition to melting point and retardation factor (Rf.).

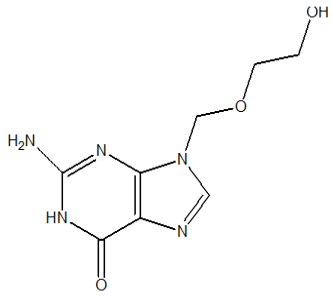
The study showed that the synthesized Schiff bases exhibited a bacterial activity that was different from acyclovir.

RECOMMENDATION

1. Study the toxicity of the compounds in order to use them as drugs.
2. The compounds may have other activities needed to be studied as antiviral activity

1-1 INTRODUCTION

Aciclovir (**ACV**), also known as **acyclovir**, is an antiviral medication.[1]It is primarily used for the treatment of herpes simplex virus infections, chickenpox, and shingles.[2] Other uses include prevention of cytomegalovirus infections following transplant and severe complications of Epstein-Barr virus infection.[2][3] It can be taken by mouth, applied as a cream, or injected.[2]



Acyclovir
2-amino-9-((2-hydroxyethoxy)methyl)-1,9-dihydro-6H-purin-6-one

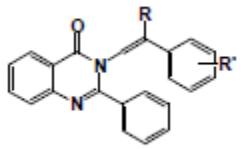
Schiff base are chemical products carrying imine or azomethine –C=N- acquired this name from German chemist's Hugo Schiff in 1864.They are produced by reacting the aldehyde or ketone with primary amines (1). They can be used as reactive intermediates for the synthesis of many neutral products (2). Schiff bases are reported show a wide range of pharmacological activities including antimicrobial, antibacterial, antifungal, antioxidant, anticancer agents (3,4). Pharmacological activities attributed by Schiff base are mainly due to characteristic C=N functionality ,synthetically, condensation of amine with carbonyl compound occurs under reflux condition with complete remove of water molecules .It also could be used for the immobilization of enzymes(5,6).Schiff base have been utilized as synthase in the preparation of a number of industrial and biological active compound like 4-thiazoldines,benzoxazines (2,3).Schiff base derivatives in various process promoted the researchers for designing of novel heterocyclic /aryl Schiff base for development of new environmental –friendly technology.

1-2 APPLICATIONS OF SCHIFF BASES ACTIVITY

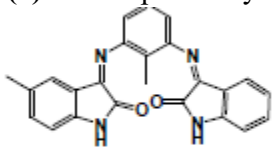
Schiff base have many applications in medicine such as

1-2-1 Antiviral activity

Schiff bases can play a vital role due to their reported antiviral nature. Schiff bases derived from isatin and bisisatin are reported to show activities against different strains of viruses [12]. Schiff bases derived from prodrug abacavir (Ziagen) were reported as anti-HIV therapy [13]. Furthermore, Schiff bases of 2-phenylquinazoline-4(3)H-one was reported to show antiviral activity against some strains of viruses like feline corona virus, influenza viruses, and herpes simplex virus [14].Salicylaldehyde Schiff bases derived from 1-amino-3-hydroxyguanidine tosylate act as new antiviral agents [15]. Isatin Schiff base ligands are marked by antiviral activity, and this fact is very useful in the treatment of HIV [16]. In addition, it was also found that these compounds have anticonvulsant activity and may beincluded in the anti-epileptic drugs [17]. Gossypol derivatives also present high antiviral activity. Increasingly, gossypol, often used in medical therapy is replaced by its derivatives, because of their much lower toxicity. Kumar *et al.* [18] reported antiviral activity of 2-hydroxy substitution on a series of 3-(benzylideneamino)-2-phenylquinazoline-4(3H)-one(2). Some bis-Schiff bases of isatin, benzylisatin, and 5-fluoroisatin **(2)** were reported by Jarrahpour *et al.* [91] as antiviral agents.



(1)



(2)

1-2-2Antiinflammtary activity and analgesic activity;

Schiff base containing sydnon that is 3-[1-(4-isobutyl phenyl) ethyl]-4(3-substituted-4-sydnonylidene)amino-5-mercapto-1,2,4-trizole was synthesized and screened for their anti-inflammatory and analgesic activity (42).

1-2-3Antidepressent activity;

Schiff base of isonicotinoyl hyrazone ,N-[IZ (substituted aromatic)methylidene]pyridine-4-carbhydrazides] were synthesized by green route of microwave and sonication, these compound have antidepressant and nootropic activity.

1-3 APPLICATION OF METAL COMPLEX SCHIFF BASE

synthesis of metal complex attained owing their versatile coordination behaviors and in the understanding of molecular process 1,2 .Metal complex are of significant attention it terms of it is structural and coordination chemistry .They display diverse chemical ,optical and magnetic properties by tailoring with different ligand ,in specific the study of metal complex of Schiff base liganed appears to be fascinating in term of

unusual structure and stability .Schiff base complexes are considered to be among the most important stereochemistry models .In transition metal coordination chemistry due to their properties accessibility and structural variety(3,4) .Transitional metal complex which usually contain nitrogen ,sulpher ,or oxygen has ligand atom have become increasingly important because these Schiff base (SB) can bind with different metal center involving various coordination sites and allow successful synthesis of metal complex 6.The interaction of these donor ligands and metal ions give complex at different geometries and literature and savory reveals that these complex are biological actives compound ,Thus recent years SB and their metal complex have attained much attraction because of their extensive biological activities (8,9).

2-1 PROCEDURE

2-1-1Extraction of acyclovir

Acyclovir (ACV) was extracted from ACYCLOVIR tablets.
Supplied by **actavis** ,UK. 10 tablets of acyclovir were crushed and 200ml of ethanol(0.2 mg/ml at 25 °C); was added then heated and filtered .Filtration was evaporated and dried at room temperature, the obtained substance was white fine crystals of acyclovir. The measured melting point = 155°C was compared with literature melting point =156-157 °C.

2-1-2-Synthesis of Schiff base of acyclovir with 4-hydroxy benzaldehyde

To (0.001mol) of acyclovir (0.225g) 40ml of ethanol (96%) was added (0.001mol) of 4-hydroxy benzaldehyde (0.122g) were mixed in 250ml round bottom flask. To the reaction mixture (4-5drops) of glacial acetic acid were added as catalyst. The reaction was reflexed with stirrer for 12 hours and the time reaction was tested by TLC the mixture was cooled overnight. The precipitate was filtered and purified by recrystallization from methanol its result are shown in table (2-1).

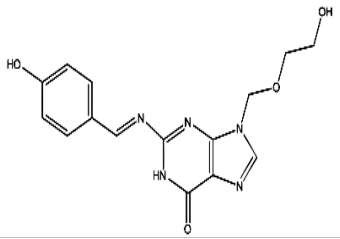
\2-1-3 Synthesis of Schiff base of acyclovir with 2-hydroxy-4-methoxy benzaldehyde

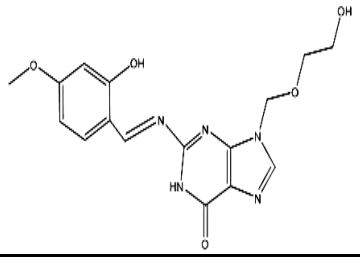
To (0.001mol) of acyclovir (0.225g) 40ml of ethanol (96%) was added (0.001mol) of 2-hydroxy-4-methoxy benzaldehyde (0.152g) were mixed in 250ml round bottom flask. To the reaction mixture (6-8drops) of glacial acetic acid were added as catalyst. The reaction was reflexed with stirrer for (9-12) hours and the time reaction was tested by TLC the mixture was cooled overnight. The precipitate was filtered and purified by recrystallization from methanol its result are shown in table (2-1).

Table (2-1) the physical properties of Schiff bases.

IUPAC Name	Reactant compounds	MP (C°)	Color and crystal form	Yield %	TLC	RF	Time of reaction
(E)-2-((4 hydroxybenzylidene)amino)-9-((2-hydroxyethoxy)methyl)-1,9-dihydro-6H-purin-6-one	Acyclovir +4-hydroxy benzaldehyde	104-106	brown crystal	60%	Chloroform: ethanol 7:3	0.60	**12 hrs.**
(E)-2-amino-9-((2-hydroxyethoxy)methyl)-1,9-dihydro-6H-purin-6-one	Acyclovir +2-hydroxyl- 4-methoxy benzaldehyde	76-77	Creamy crystal	73%	Hexane: ethanol 7:3	0.36	**10 hrs.**

Table (2-2) The symbol, name and structure of Schiff bases.

Symbol	Name of compound	Structure
1	(E)-2-((4 hydroxybenzylidene)amino)-9-((2-hydroxyethoxy)methyl)-1,9-dihydro-6H-purin-6-one	

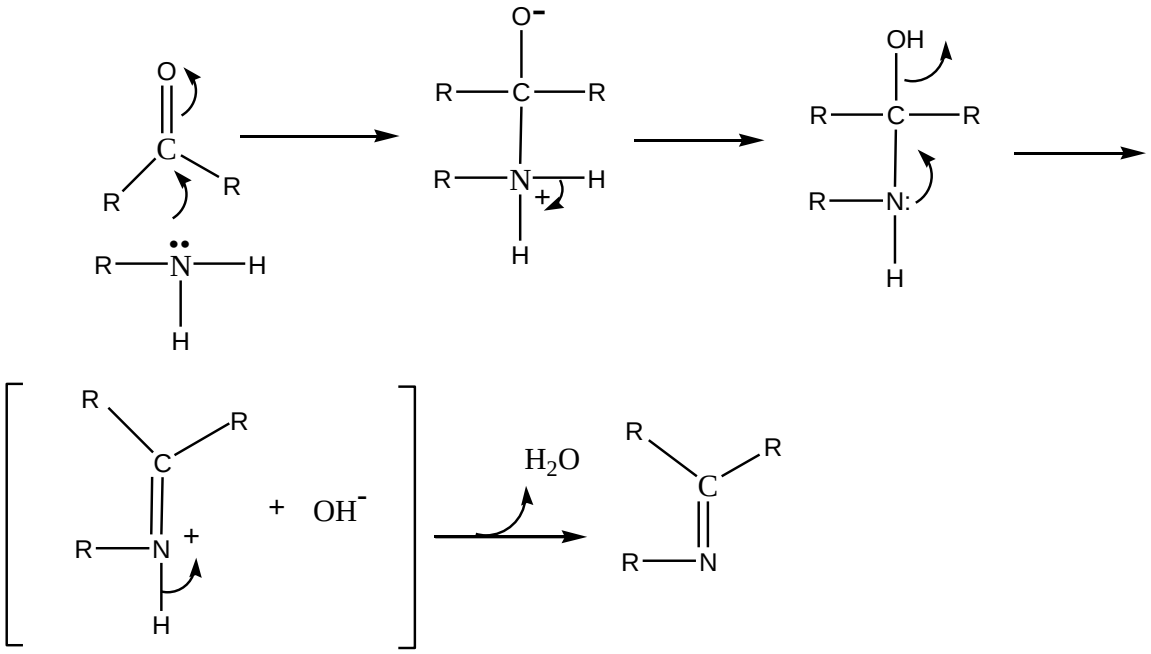
2	(E)-2-amino-9-((2-hydroxyethoxy)methyl)-1,9-dihydro-6H-purin-6-one	

DISCUSION

3-1 *INFRARED SPECTRA OF SCHIFF BASES*

The different Schiff bases were prepared and studied in this research. Schiff bases were formed by the condensation reaction of some aldehydes such as 4-hydroxy benzaldehyde, 2-hydroxy-4-methoxybenzaldehyde with acyclovir drug in absolute ethanol with elimination of water at the end of the reaction, as shown in scheme (3-1).

The mechanism of the reaction, as illustrated in scheme (3-1), included the nucleophilic attack of an amine at the carbon atom of carbonyl group.



Scheme (3-1) **The mechanism of the formation of Schiff base.**

The intermediate form throughout the reaction undergoes elimination of water to yield the appropriate Schiff base. The yield of products depends on the type of substituted groups. Therefore, the presence of electron donating groups in amine and electron withdrawing groups in an aldehyde increases the yield of products or vice-versa. As an example, the yield of **2** was 73% more than **1** 60%.

The disappearance of the absorption peaks may be attributed to the carbonyl group which presence in the aldehyde compounds in the IR spectra indicating the complete participation in the condensation reactions.

The IR spectra of the synthesized Schiff bases are shown Figures (3-1) and (3-2). The data of the most important absorption bands for 1 and 2 are summarized in Table (3-1).The first band appeared in the region (1647-1666 cm^{-1}) may be attributed to C=N group. The stretching vibration of C=N group could not be identified individually due to nearness to the stretching vibrations of C=C.

For aliphatic C-H bond, infrared spectra of 1and 2 exhibited absorption bands in the range (2850-2854 cm^{-1}) which are attributed to the symmetrical stretching vibration.

Table (3-1) the main peaks of FT-IR spectra (cm^{-1}) of Schiff bases compounds.

Symbol	C=N	C=C	Ar-H		H-C=N	Alphatic C-H	−OH
	(Str.)	(Str.)	Str.	Bend. O.O.P	Str.	Str.	(Str.)
1	1660	1639 1504	3024	837	2924	2854	3549
2	1647	1589 1568	3043	891	2931	2850	3549

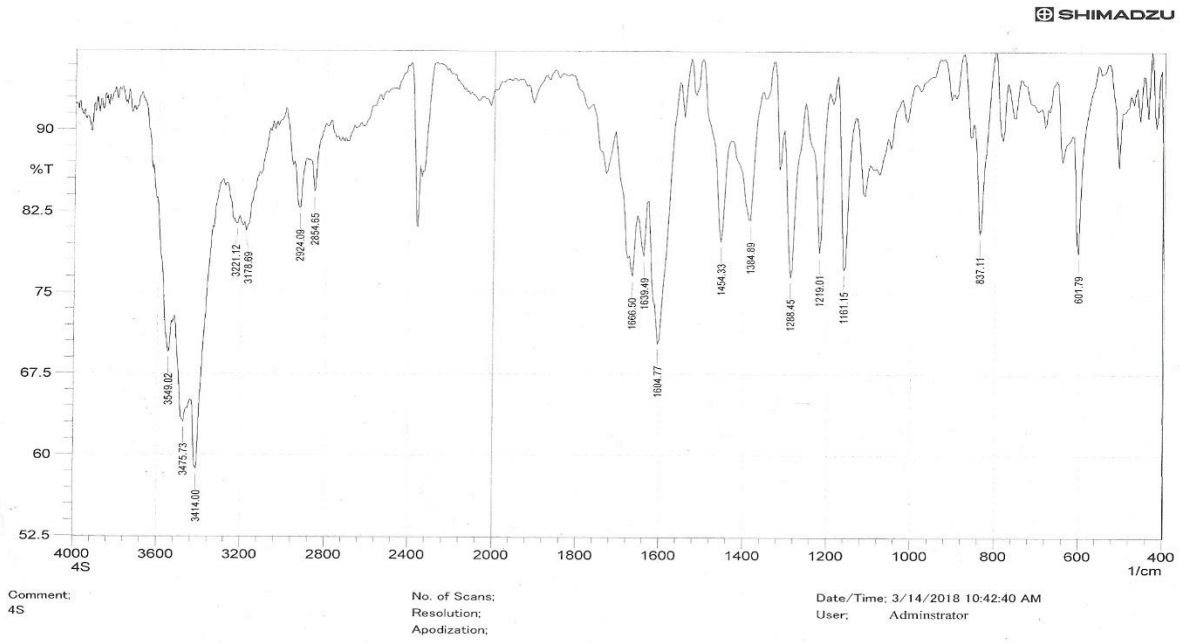


Figure (3-1) FTIR spectrum of compound 1.

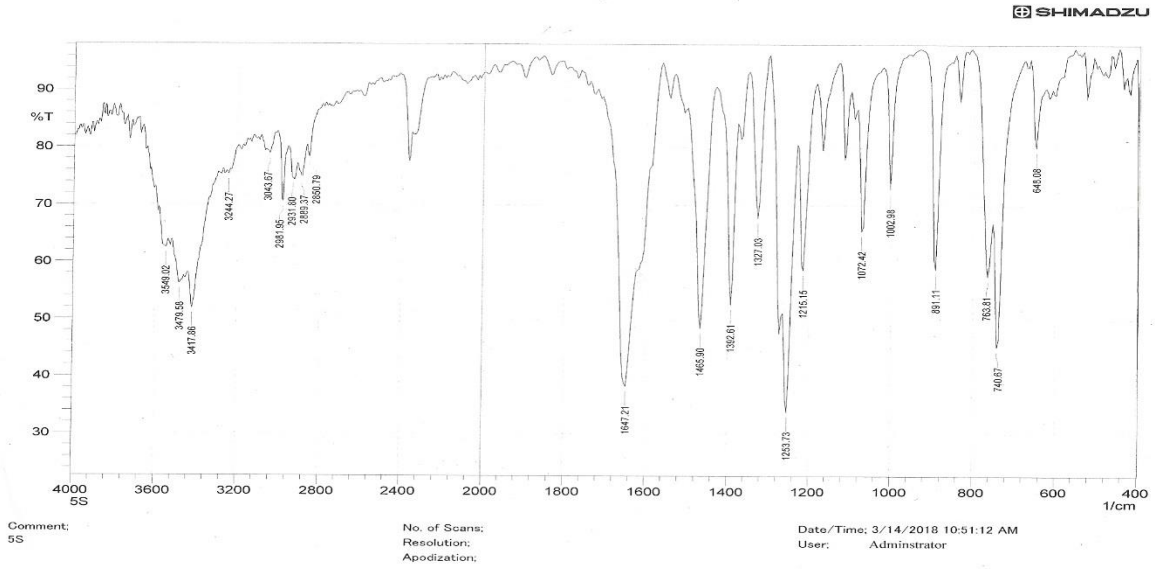


Figure (3-2) FTIR spectrum of compound 2.

3-2 Bacterial activity

3-2-1 five types of bacterial species were used:

1- Gram-positive Bacillus.
2- Gram- negative Proteus.
3- Gram-negative *Escherichia coli* clinically isolated.
4- Gram- negative Pseudomonas.
5- Gram- negative Klebsiella.

3-2-2 The biological activity of Schiff base compounds

Kirby Bauer process was used with discs (6 mm diameter) of filter paper which were sterilized by the autoclave apparatus at 100 °C for 1 hr. under 1 atm. and kept in clean sterilized glass screw plugs. The plate agar was diffused with slight modification by using Muller-Hinton agar (M.H.A) medium and was sterilized by autoclave apparatus at 121 °C for 15 minutes under 1 atm and then cooled and poured into sterilized Petri dishes 11 cm.

Five bacterial kinds were used, Gram positive *Bacillus* and Gram negative *Escherichia coli* (*E- Coli*), *Proteus*, *Pseudomonas* and *Klebsiella.*
The isolated bacteria were cultured by streaking on nutrient agar (N. A.) in order to obtain youth colonies of 24 hours age. The discs saturated with the synthesized compounds (conc. 0.009 mg/ml) in DMSO (Dimethyl sulphoxide) were added to (M.H.A.) medium by clean forceps, and then incubated at 37 °C for 24 hours.

The dishes were examined for the presence or absence of bacterial growth and the averages of inhibition zone diameters around each disc in millimetres were measured (the zone where there is no bacterial growth, as shown in Table (3-2).

The antibacterial activities of the synthesized compounds against the tested organisms; *Staphylococcus aureus* and *Escherichia coli* using Hahn method are summarized in Table (3-2) and Figure (3-1) to (3-3). This method was based on the disc diffusion for testing chemical agents and antimicrobial efficiency by measuring the agent's zones of inhibition whose sizes are relative to the sensitivity of the organism to the particular antibiotic in the disc.

Table (3-2) Inhibition Zones (IZ) in millimetre (mm) of the synthesized compounds.

Compounds	Ionization zones in mm				
DMSO (solvent)	*Bacillus*	*Proteus*	*E- Coli*	*Pseudomonas*	*Klebsiella*
***1**	10	10	9	8	** -
***2**	30	28	14	12	15

* 1, 2 Schiff bases of acyclovir drug
** - Lack of microbial growth inhibition

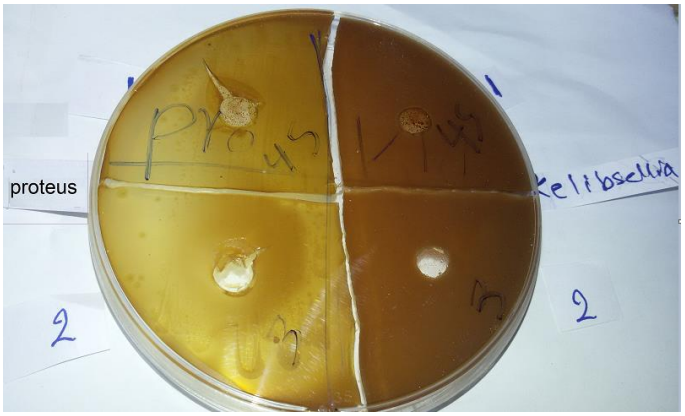


Figure (3-1) Inhibition Zones of 1 and 2 against *proteus* and ***Klebsiella* .**

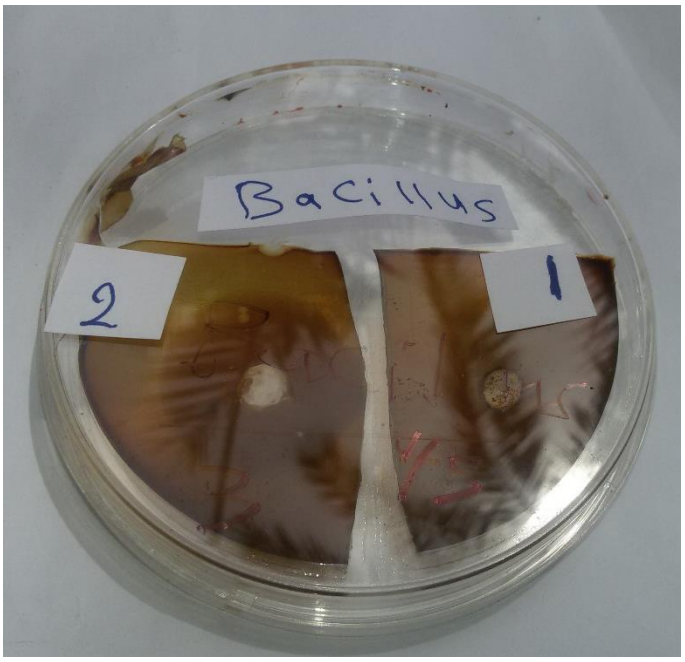


Figure (3-2) Inhibition Zones of 1 and 2 against *Bacillus.*

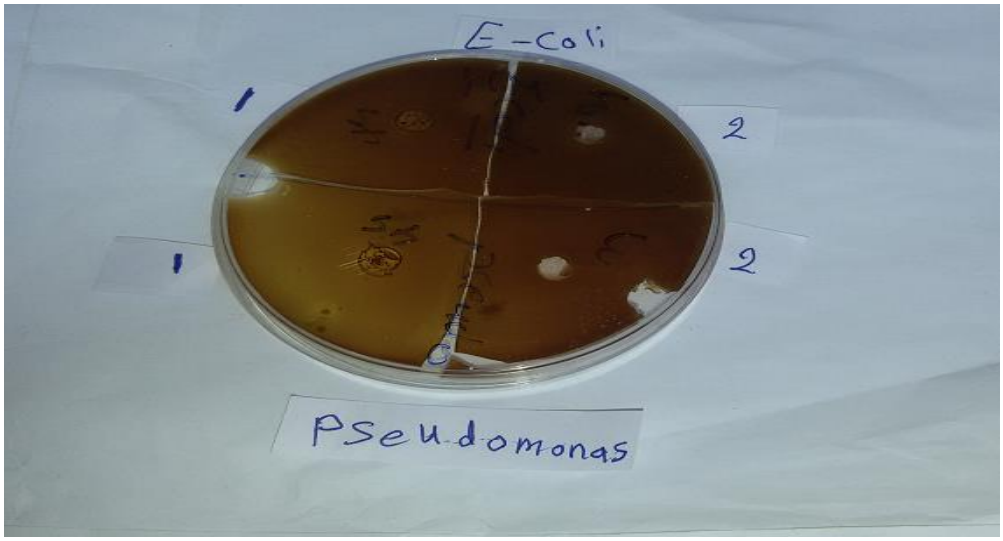


Figure (3-3) Inhibition Zones of 1 and 2 against *E-Coli* and *Pseudomonas*.

From this result, The Schiff base **2**, prepared from acyclovir with 2-hydroxy-4-methoxy benzaldehyde, showed high activity on Gram positive bacteria *Bacillus* with inhibition zone 30 mm and on Gram negative bacteria *Proteus* with inhibition zone 28mm than Schiff base **1**, prepared from acyclovir with 4-hydroxy benzaldehyde with inhibition little activity against these. Also, showed high activity with other Gram negative bacteria like *E- Coli, Pseudomonas , Klebsiella.*This may be because the structure of Schiff base **2** contain addition methoxy group compare with **1**.

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