*Synthesis And Identification New Types From Antimicrobial Polymers And Study Biological Activity

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Abstract :

This research describes the synthesis new Homo polymers and new co-polymers based on synthesis of Oximes as monomers which can synthesized from the reaction of (p-Hydroxyacetophenone , and p-Nitroacetophenone**),** with Hydroxylamine hydrochloride in presence of NaOH, *The* Homo polymer was synthesized from the reaction of oxime with each others and formaldehyde in presence of HCl as catalyst,While the co-polymers were prepared from the reaction of oxime with other monomers such as (Toluen sulphonic acid , 2,4-Di chloro benzoic acid) and formaldehyde in presence of HCl as catalyst,These polymers were identified by FT-IR and ^{1}HNMR spectroscopy, The antibacterial,antifungal and antiyeast activities of the synthesized polymers were also screened on various bacteria ,fungal and yeast .All the prepared polymers show excellent antimicrobial activities as compared to the standard ciprofloxacin and amphotericin –B drugs.

Keyword: antimicrobile , Polymers ,biodegeradable , Polymers , antimicrobile , biodegeradable

Chemistry classification : QD 241- 441

Introduction:

Polymers have been used for decades instead of metal, glass and wood in many applications due to their superior physicochemical properties, in addition to others, as well as for reasons of economy. For example, commercially available thermoplastic polymers, such as polyolefins, are hydrophobic and biologically inert. This has made them indispensable in the packaging industry, distribution of food stuffs and other perishable commodities [1]. Another example is agriculture, where plastics have largely replaced glass in the construction of green houses, in addition to which they have gained a unique position in the growing of soft fruit and vegetables over mulching films [2]. Increasing demands on polymer materials have led to their further development. In some applications, the polymer products also possess, besides the passive function (e.g. packaging or structural) an active function (e.g. protective and/or indicative). The polymeric materials with resistance to microbial colonization and pathogenic

***The Research is apart of on M.Sc. thesis in the case of the Second researcher**

microorganism spreading (antimicrobial polymers) have been one of the examples of the active material functionality. The antimicrobial polymers are expected to protect against negative impact of the pathogenic microorganisms, which can seriously affect the society from the viewpoint of both health damages and unwanted economical loads connected with that [3].

Antimicrobial polymers are environmentally friendly in that potentially toxic chemicals are not incorporated and hence cannot leach out.[4] Furthermore, they are easily incorporated into fibers, extruded to fibers or electrospun into nanofibers and prevent adhesion of microorganisms to their surface. Antimicrobial polymers are synthesized by covalent bonding of biocidal functional groups in a post-polymerization modification, providing antimicrobial or antiseptic properties.[5]Modification is either to the bulk polymer or selectively to the surface via available reactive moieties.[4] Another form of synthesis is the chemical modification of a biocidal molecule into a polymerizable compound that can subsequently be polymerized or co-polymerized with another monomer.[6–7]

Researchers around the world have learnt to modify the already known low molecular weight antimicrobial agents, which are proven to have lethal actions against a broad spectrum of microorganisms.[8]

This has probably helped to consider materials with bioactive functionalities as a start. Screening the organic biocides for example, one would find numerous molecules that could be incorporated and modified to be immobilized to the bulk polymer or on the surface.[9]

Many factors have found to affect the antimicrobial efficacy of these materials, such as molecular weight of the polymer, spacer length between active site and the backbone, hydrophobic tail length attached to the active site, and the hydrophilic-hydrophobic balance of the material. [10]

In this research is to prepare the new antimicrobial polymers based on Oximes (monomer) to produce two types of polymers , Homo polymer and co-polymers .

Then synthesized polymers are examined against different types of microorganisms includes Bacteria, Fungi and Yeast,his study was to find a new antimicrobial polymers to inhibit the growth of pathogenic microorganisms which can used in many application.

2. EXPERIMENTAL

2.1 Materials

Hydroxylamine hydrochloride(ALDRICH), p-Nitroacetophenone (ALDRICH), . Formaldehyde (ALDRICH), NaOH (BDH) **,** HCl (HIMEDA), 2,4-Di Chlorobenzoic acid (HIMEDA) **,** 5-Sulphosalicalic acid (MERCK), P-Hydroxyacetophenone . (MERCK), Toluen sulphonic acid (MERCK).

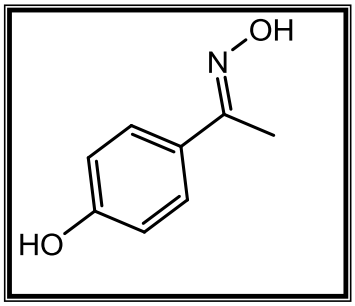
2-2 .*Apparatus*

(FTIR) Forier Transform infrared spectrophotometer 4800S ,Shimadzu,Jaban, (Hot plate stir) Bibby Strlintd , (Recorded NMR spectra) using atype of Bruker,Ultra shield 300Mhz,Switzerl and using (DMSO-d^6) as a solvent at the university Al-Albyt in the Hashemite Kingdom of Jordan.

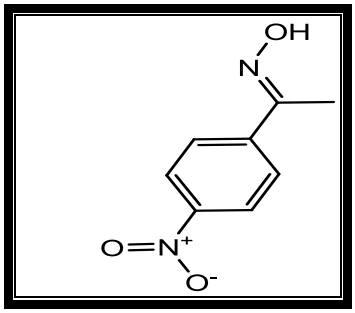
2.3 Methods

2.3.1 Monomer synthesis

(5.0 g) (0.074 mol)of hydroxylamine hydrochloride was dissolved in(10.0 ml) of water in a conical flask and a solution of (3.0 g)(0.075 mol) of NaOH (in 10 ml water) was added to it. The solution was cooled and (6.0 g) of P-Hydroxyacetophenone was slowly added to the solution. The flask was then cooled, shaken well and left overnight so as to get the crystals of oxime. The crystals were filtered at the pump and dried rapidly by pressing between filter papers . The other monomers (Oximes)were prepared by the same procedure as above procedure.[11]



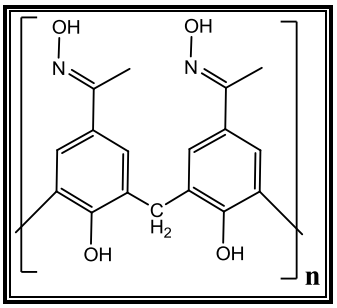
Scheme (2 – 1) Structure of P- Hydroxyacetophenone oxime .



Scheme (2 – 2) Structure of P- Nitroacetophenone oxime .

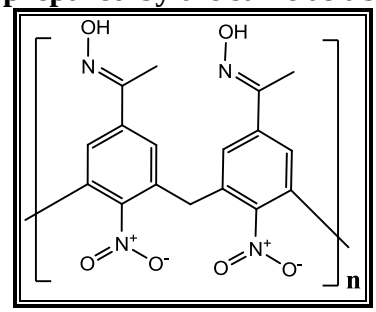
2.3.2. Homo Polymer synthesis :

A mixture of p-Hydroxyacetophenone oxime (0.01 mol) (1.66 gm) , and condensing reagent formaldehyde (0.02mol) were taken in a round bottom flask. (200 ml) of (2 M) HCl was added slowly to the reaction mixture and the contents were refluxed to 120 - 130°C for 8-10 hours on an oil bath with periodic shaking. After completion of the reaction, the mixture was extraction by diethyl ether and purified by dissolved in (8%) NaOH solution then filtered . Finally, the product was washed with hot water to remove unreacted reactants , and dried in vacuum.[11]



Scheme (2 – 3). Structure of PHAO1.

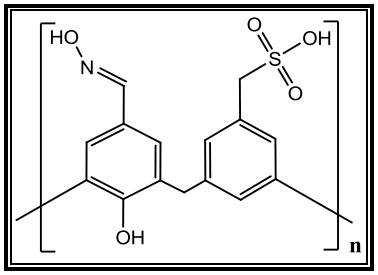
The other polymers were prepared by the same as above procedure .



Scheme (2 – 4). Structure of (PNAO1).

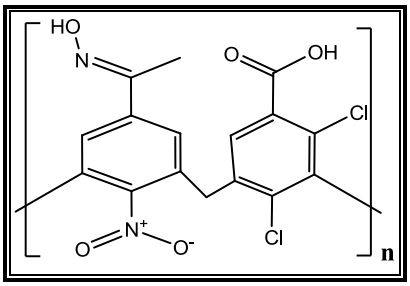
2.3.3. Hetero polymer synthesis :

A mixture of p-Hydroxyacetophenone oxime (0.01 mol), formaldehyde (0.02mol) and the co-monomer (Toluen sulphonic acid) (0.1 mol) (0.88 gm) were taken in a round bottom flask.and 200 ml of (2 M) HCl was added slowly to the reaction mixture and the contents were refluxed to 120 - 130°C for 8-10 hours on an oil bath with periodic shaking. After completion of the reaction, the mixture was extraction by diethyl ether and purified by dissolved in (8%) NaOH solution then filtered . Finally, the product was washed with hot water to remove unreacted reactants , and dried under vacuum pump.[13]



Scheme (2-5) Structure Of (PHAO2) .

The other polymers were prepared by the same as above procedure .



Scheme(2 -6)Structure Of (PNAO2) .

2.4. Antibacterial activities

Pure cultures of pathogenic bacteria *viz*. *Escherichia coli, klebsiella pneumoniae, Staphylococus aureus, salmonella typhi* ,and *Pseudomonas aeurginosa* were used for antibacterial activity. Cup or well method was used for antibacterial studies. Nutrient agar medium was used for culture of the bacteria. The composition was beef-extract (3.0 g), peptones (5.0 g) sodium chloride (5.0 g) agar-agar (15.0 g) and distilled water (1000 ml). Nutrient agar medium was autoclaved at 15 psi and 121oC for 15 minutes. Sterilized Petri dishes were placed in laminar flow bench. One end of the lid of each petri dish was lifted and approximately 15- 20 ml of molten agar medium was poured into it and left for solidification. These were then inoculated with 0.2 ml suspension of organism by spread plate method. With the help of sterile borer, 2-4 wells ,were made in the medium and subsequently peripheral wells were filled with 500 ppm solution of synthesized plolymers and central well was filled with the standard drug used i.e. ciprofloxacin at the same concentration. Other petri dishes were sealed with paraffin and incubated at 37º C in an incubator. The petri dishes were examined for zone of inhibition after 24-48 hours. Concentrations of samples for antibacterial activity were taken as 500 µg/ml .[17]

2.5. Antifungal and Yeast activities

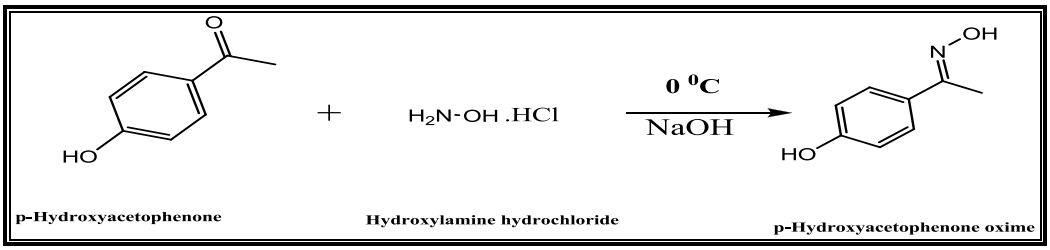
Pure cultures of pathogenic fungi *viz*.(*Alternaria solani, Fusarium oxeyspurum , Aspergillus niger , and Mucor) and the Yeast is (candida albicans , candida krusei , candida parapsilosis , and candida tropicalis)* were used for antifungal activity studies. Antifungal activity of the synthesis polymers was evaluated using poisoned food technique on potato dextrose agar (PDA) medium. In this method, 20 ml of potato dextrose agar medium was poured in sterilized Petri plates along with 1.0 ml of synthesis polymers (1.0 mg/ml) and plated 6 mm diameters cups were removed from the centre in which the same diameter mycelial discs (7 days old culture) were inoculated. PDA medium without extract served as a control and the percent inhibition of fungal growth was determined by comperd with standard drug .[17]

Results & Dissections:

3.1. Synthesis and Characterization of monomers:

3.1.1 Synthesis and Characterization of (Oxime 1)

The (Oxime 1) was synthesized from the reaction of p-Hydroxyacetophenone with Hydroxylamine hydrochloride in presence of NaOH then the solution was cooled , shaken well and left overnight . this reaction was shown in Scheme (3.1). [17]



Scheme 3.1

In Figure (3.1) The FTIR spectra of (Oxime 1) shows the absorption band of C=C Aromatic at 1604cm^{-1}, O-H at 3325 cm^{-1}, C=N at 1665 cm^{-1}, N-O at 941 cm^{-1}C-H aromatic at 3055 cm^{-1}, C-H at 2792 cm^{-1} , and C-O at 1242 cm^{-1}.

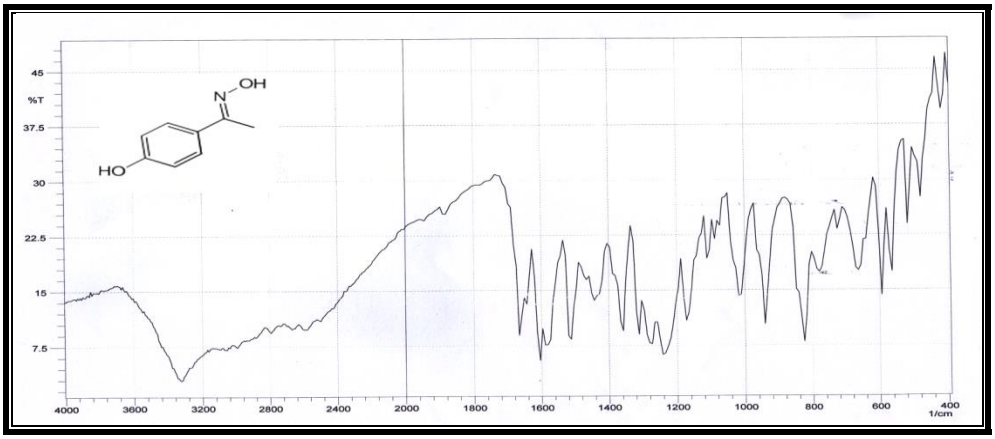
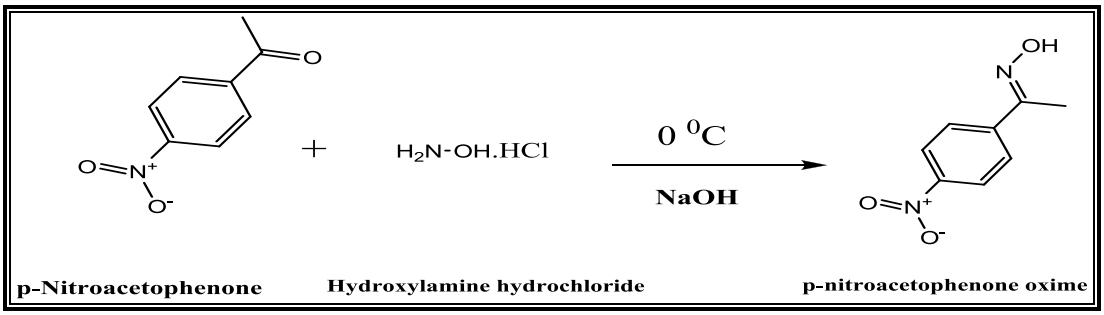


Figure (3.1) The FTIR spectra of (Oxime 1)

3.1.2 Synthesis and Characterization of (Oxime 2)

The (Oxime2) was synthesized from the reaction of p-Nitroacetophenone with Hydroxylamine hydrochloride in presence of NaOH then the solution was cooled , shaken well and left overnight . this reaction was shown in Scheme (3.2). [17]



Scheme 3.2

In Figure (3.2) The FTIR spectra of (Oxime 2) shows the absorption band of C=C Aromatic at 1595 cm^{-1}, C-H Aromatic at 3145cm^{-1}, OH at 3400 cm^{-1}, C= N at 1650 cm^{-1}, and N=O at 1550 , C-H aliphatic 2923 cm^{-1} , and 1345 cm^{-1}.

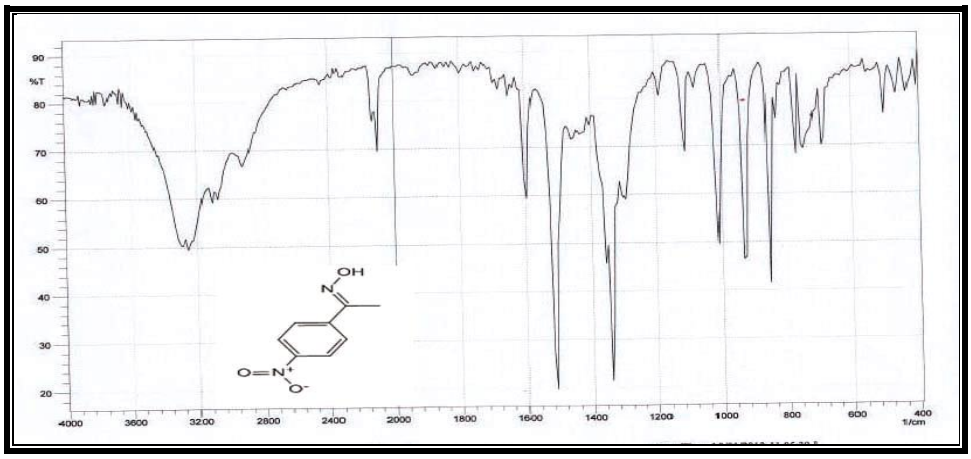
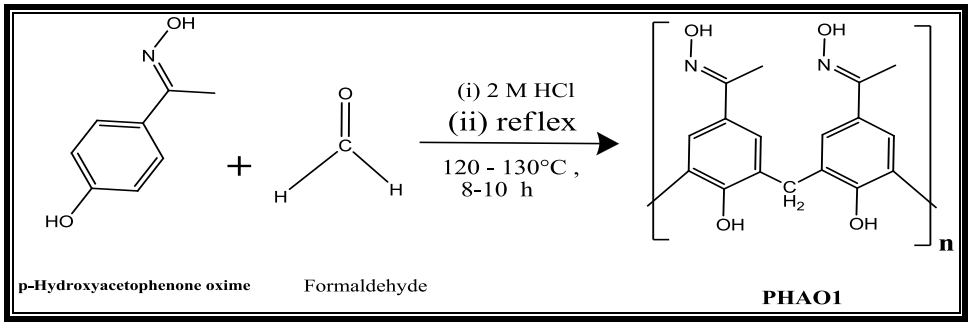


Figure (3.2) The FTIR spectra of (Oxime 2)

3.2. Synthesis and Characterization of Homo Polymers:

3 .2.1 Synthesis and Characterization of (PHAO1):

The (PHAO1) was synthesized from the reaction of P-Hydroxyacetophenone oxime and formaldehyde in presence of HCl as catalyst by refluxing for 8 – 10 hrs.this reaction was shown in Scheme (3.3). [17]



Scheme 3.3

In Figure (3.4) The FTIR spectra of (PHAO1) shows the absorption band of C=C Aromatic at 1598cm^{-1}, O-H at 3200 cm^{-1}, OH phenolic at 3670 cm^{-1}N=C 1695 cm^{-1}, N-O at 990 cm^{-1} , C-H aliphatic at 2970 cm^{-1} C-H Aromatic at 3178 cm^{-1}, and C-O at 1350 cm^{-1}.

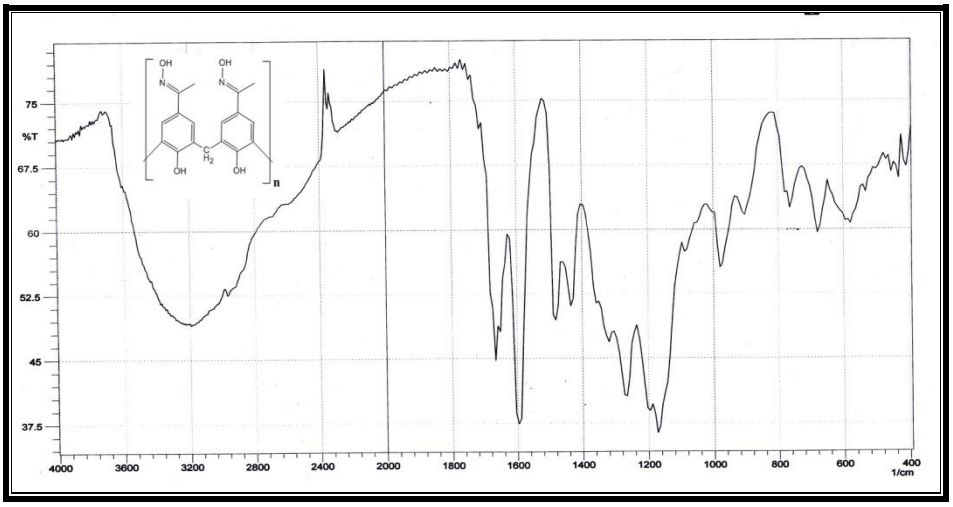


Figure (3.4) The FTIR spectra of (PHAO1)

The ^{1}HNMR spectra of (PHAO1) **,**shown in figure (3.5), assign the following chemical shifts : δ (2.135) ppm (S, 2H) for N-OH group , δ (3.372) ppm (S, 6H) for CH3 group , δ (5.405) ppm (S ,2H) for OH group ,δ (7.397) ppm (S, 4H) for ph-H group ,δ (4.372) ppm (S, 2H) for CH2 group .

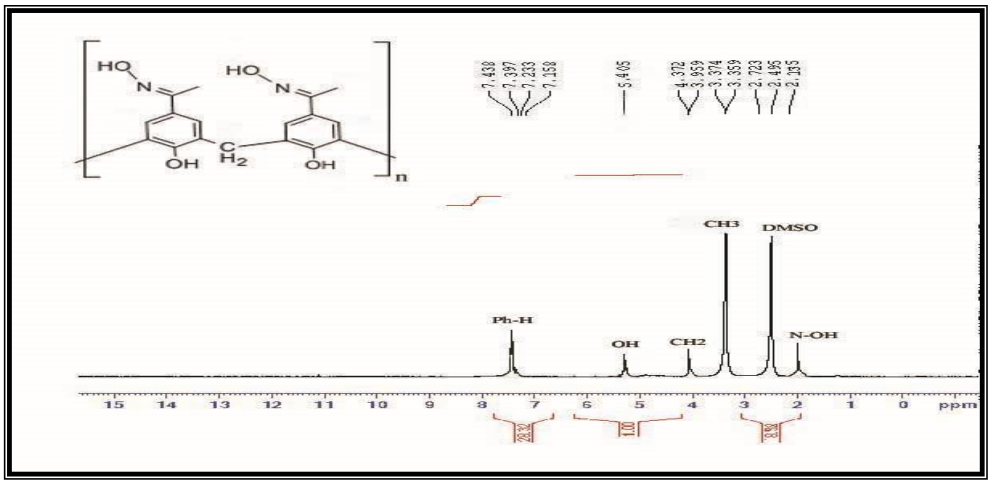
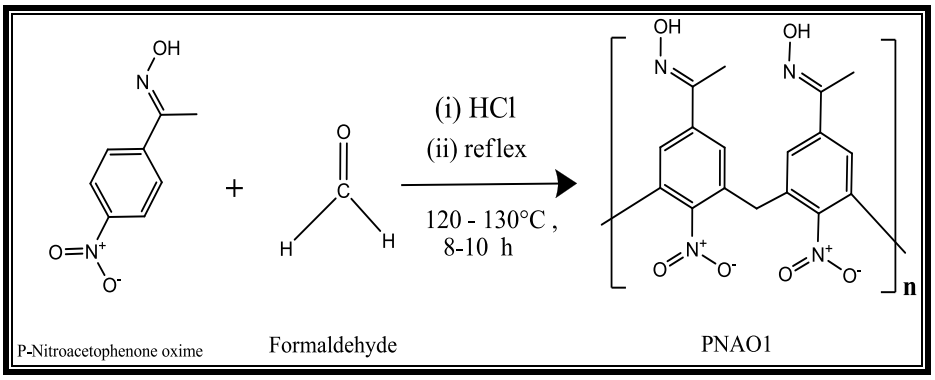


Figure (3.5) The ^{1}HNMR spectra of (PHAO1) .

3.2.2 Synthesis and Characterization of (PNAO1):

The (PNAO1) was synthesized from the reaction of P-Nitroacetophenone oxime and formaldehyde in presence of HCl as catalyst by refluxing for 8 – 10 hrs.this reaction was shown in Scheme(3.4). [17]



Scheme 3.4

In Figure (3.6) The FTIR spectra of (PNAO1) shows the absorption band of C=C Aromatic at 1600 cm^{-1}, C-H Aromatic at 3150 cm^{-1}, OH at 3100 cm^{-1}, N=O at 1370 , 1545 cm^{-1}, C=N at 1700 cm^{-1} , N-O at 930 cm^{-1} and C-O at 1255 cm^{-1}.

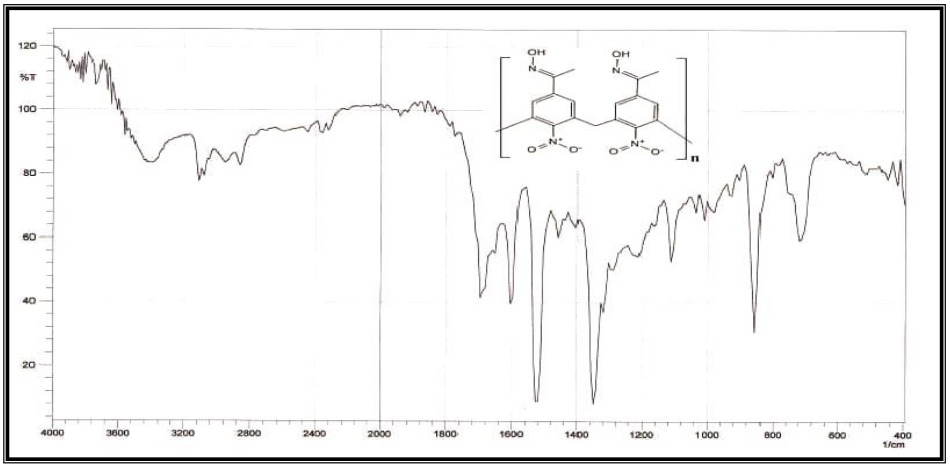


Figure (3.6) The FTIR spectra of (PNAO1)

The ^{1}HNMR spectra of (PNAO1) **,**shown in figure (3.7), assign the following chemical shifts : δ (2.75) ppm (S , 6H) for CH3 group , δ (3.697) ppm (S , 2H) for CH2 group ,δ (4.953) ppm (S ,2H) for OH group , δ (8.323) ppm (M , 2H) for aromatic protons .

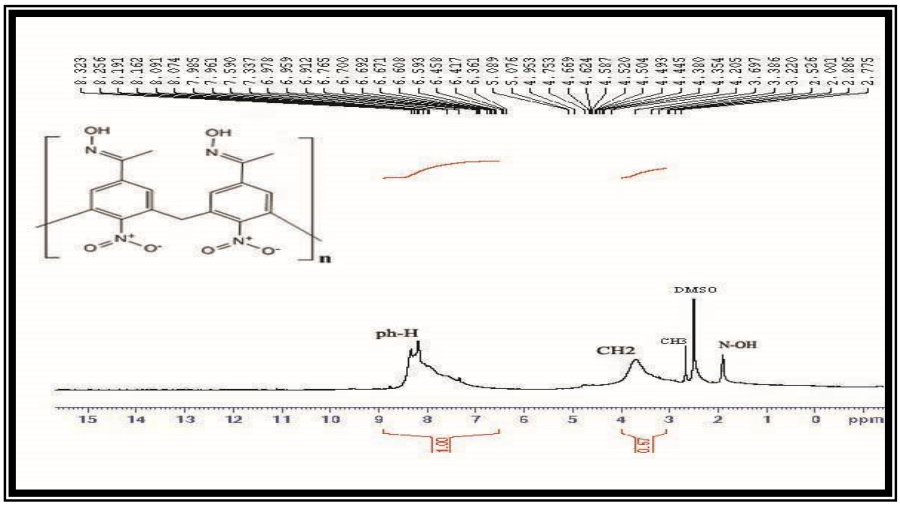
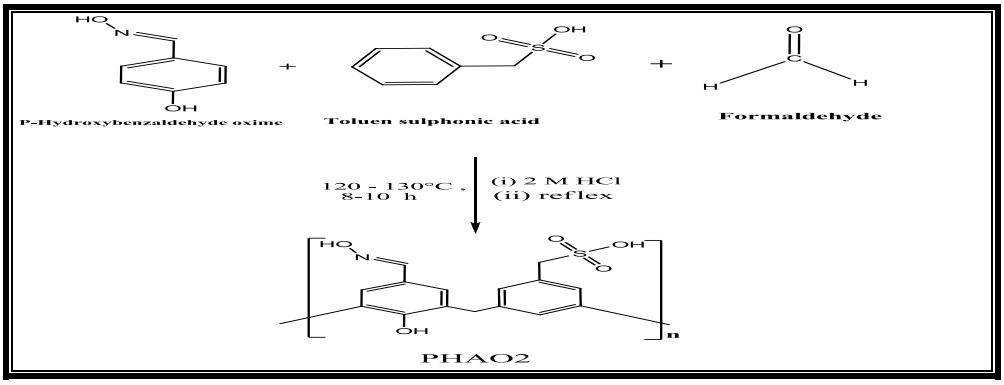


Figure (3.7), The ^{1}HNMR spectra of (PNAO1) .

3.3. Synthesis and Characterization of CO - Polymers:

3.3.1 Synthesis and Characterization of (PHAO2):

The (PHAO2) was synthesized from the reaction of p-Hydroxyacetophenone oxime with Toluen sulphonic acid and formaldehyde in presence of HCl as catalyst by refluxing for 8 – 10 hrs. this reaction was shown in Scheme (3.5). [17]



Scheme 3.5

In Figure (3.8) The FTIR spectra of ($PHAO_2$) shows the absorption band of S=O at 1530 cm^{-1} , C-S at 710 cm^{-1} , N-OH at 1510 cm^{-1} , C=N at 1620 cm^{-1} , C=C Aromatic at 1670 cm^{-1} , C-H aliphatic at 2893 cm^{-1} , C-H aromatic at 3390 cm^{-1} , and O-H at 3450 cm^{-1} .

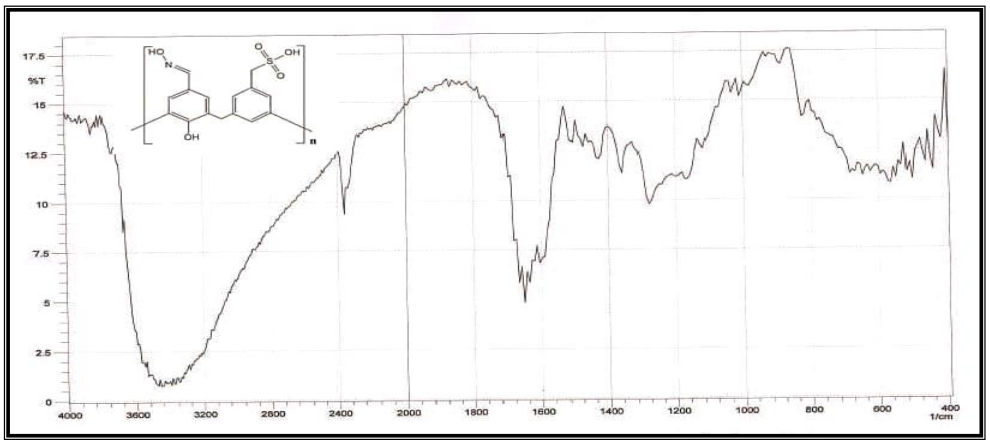
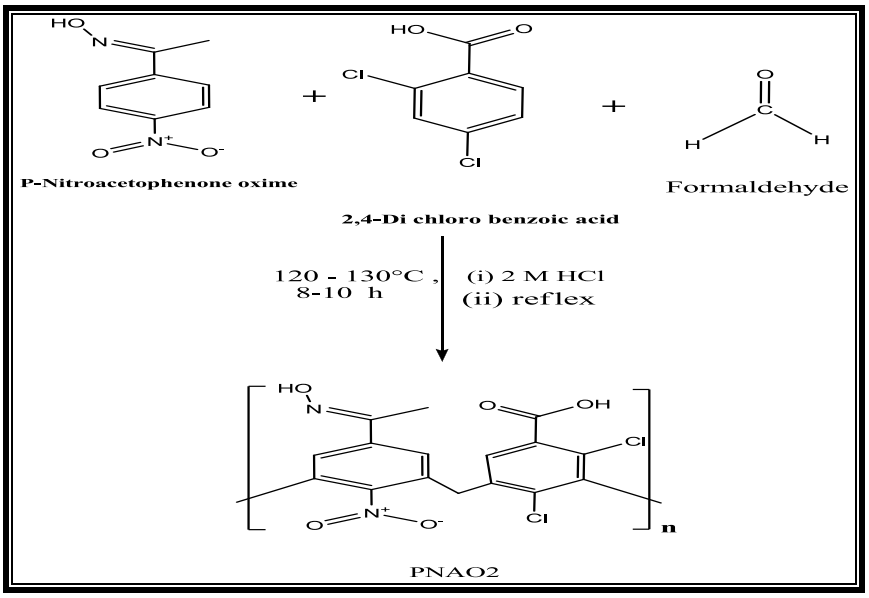


Figure (3.8) The FTIR spectra of (PHAO2)

3.3.2 Synthesis and Characterization of (PNAO2):

The (PNAO2) was synthesized from the reaction of p-Nitroacetophenone

Oxime with 2,4-Di chloro benzoic acid and formaldehyde in presence of HCl as catalyst by refluxing for 8 – 10 hrs. this reaction was shown in Scheme (3.6). [17]



Scheme 3.6

In Figure (3.9), The FTIR spectra of (PNAO2) shows the absorption band of O-H at 3450 cm^{-1} , C-H aromatic at 3100 cm^{-1} , C-Cl at 785 cm^{-1} , N=C at 1680 cm^{-1} , N-O at 980 cm^{-1} , N=O 1150 cm^{-1} , C-H aliphatic at 2939 cm^{-1},and C=O at 1708 cm^{-1}.

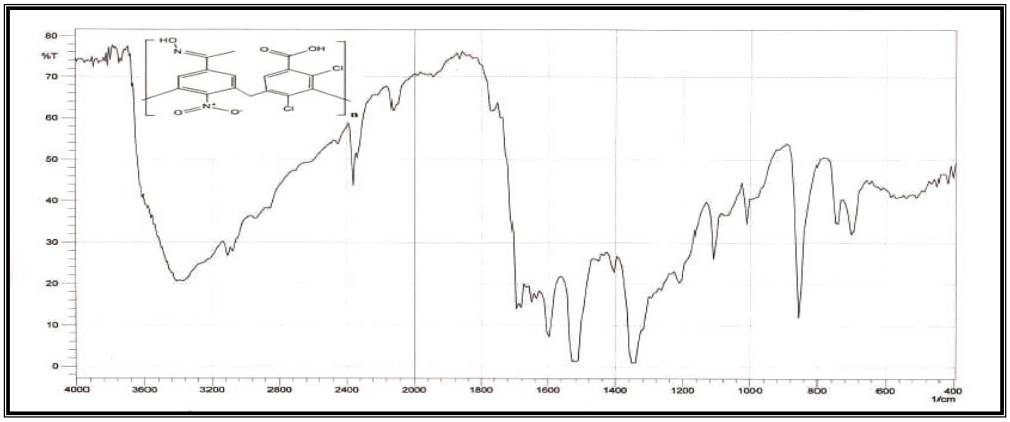


Figure (3.9) The FTIR spectra of (PNAO2)

The [1]HNMR spectra of (PNAO2) **,**shown in Figure (3.10) , assign the following chemical shifts : δ (2.146) ppm (S,2H) for N-OH group,δ (3.952) ppm (S,3H) for CH3 group ,δ (8.3) ppm (M ,3H) for Aromatic proton ,δ (11.05) ppm (S ,1H) for carboxylic protone .

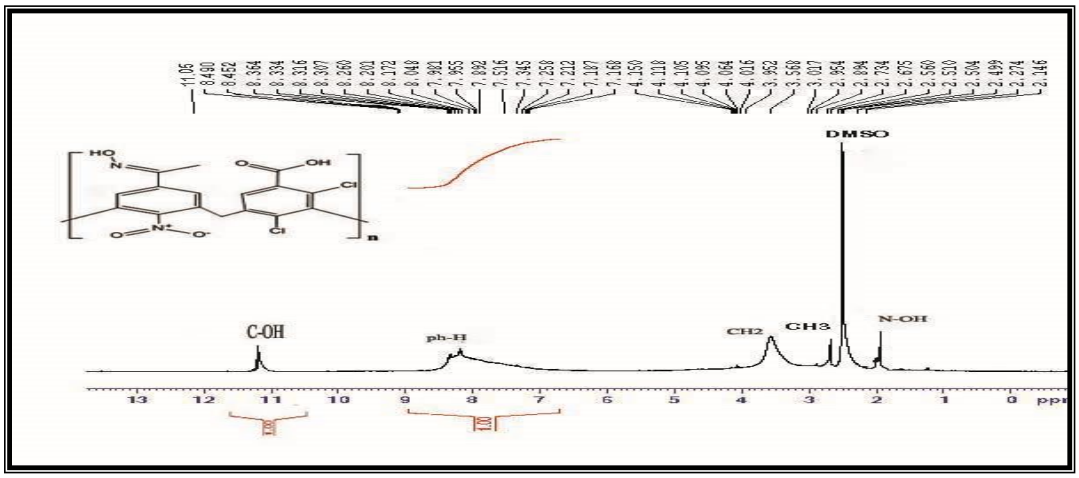


Figure (3.10) The [1]HNMR spectra of (PNAO2)

3.4- Effects of Antimicrobials on Growth of Organism

Antimicrobials are substances that kill or prevent growth of microbial cells.Some antimicrobials ,such as penicillin are derived from microorganisms ,where are used therapeutically in the treatment of diseases caused by microbial pathogens because these agents have a selective activity .That is , they interfere with some metabolic factor or process in the pathogen, but they have a little or no effect on the host. In most cases , selectivity is primarily the result of largest factor or process not being present in the host cell.[18]

Not all antimicrobial are bactericidal , that is they exert their effect by killing cells, some are only bacteriostatic and prevent growth as long as are they are present . However if the agent is removed from the culture medium of a sensitive microorganism , its effects are reversed and normal growth can resume . It should be

noted that the distinction between bactercidial, and bacteriostatic is not precise and may be influenced by the concentration of the antimicrobial agent [19].

3.5 Organisms tested

The antimicrobial activity of the synthesized polymers was tested against (*Pseudomonas aeruginosa; Escherichia coli; Klebsiella pneumoniae ;* and *Salmonella typhi)* and against fungi *Alternaria solani, Fusarium oxeyspurum , Aspergillus niger ,* and *Mucor,* against *Candida albicans , C. tropicalis , candida krusei , candida parapsilosis* which were obtained from AL-Qadisiyah University, College of Science Biology Department .

3.6. *Factor effected on antimicrobial activity based on synthetic polymers:*

The ability to successfully mimic the biochemical activity of antimicrobial polymers has been demonstrated by several groups. This has been accomplished by careful tuning of the molecule's hydrophobicity, details of membrane insertion and charge density.[19] The biological properties of polymeric synthetic mimics result from the interplay of many parameters, it is not yet possible to predict the exact properties of such molecules from their mere chemical structure. However, as demonstrated here, the effect of certain design features such as charge and hydrophobicity on the properties across a polymer series is understood. Compared to the mechanistic specifics that are known about the interactions or small antibacterial molecules with membranes and cells, relatively little is known concerning the interaction of polymeric with membranes.[20]

3.6.1 Charge effected

Microbial cells generally carry a negative net charge at the surface due to their membrane proteins, teichoic acids of Gram-positive bacteria, and negatively charged phospholipids at the outer membrane of Gram-negative bacteria. This way, polycations are attracted and if they have a proportionate amphiphilic character, they are able to disrupt the outer as well as the cytoplasmic membrane and afford lysis of the cell resulting in cell death. [21]

3.6.2 Hydrophilic/hydrophobic effected

Hydrophilicity and hydrophobicity are conceptions also based upon water ambience, upon which the manner of antimicrobial interaction of prepared polymers are determined.It was found that most polymers consist of Hydroxyl group with cationic hydrophilic groups and hydrophobic groups , which are arranged on opposite faces of the molecule, thus creating an overall facially amphiphilic architecture [22] aromatic backbone, they have no intra molecular hydrogen bonds. This allowed the repeat units to rotate around the single bonds of the backbone, and enabled them to orient their functional groups to a facially amphiphilic conformation upon contact with the cell membrane or a similar hydrophilic–hydrophobic interface.[23]

However, with increasing hydrophobicity, the polymers also become more toxic to microbial cells.

4. Biological Activity

Antimicrobial polymer is a polymer that has the ability to kill microorganisms, by acting as a source of sterilizing ions or molecules .

Generally, the use of conventional antimicrobial agents is associated with the problems of residual toxicity of these agents which can cause more serious problems to the environment. For example, in the case of using these antimicrobial agents in food packaging, there is a risk of diffusion of these agents into the food causing various problems [24]. In water treatment, the most popular treatment method to disinfect and sterilize water is to use chlorine and other related chemicals. However, their residues can become concentrated in the food chain and in the environment as well as the possible formation of halomethane analogues that are suspected of being carcinogenic should lead to the avoidance of their use [25].

The chemical structure of the prepared polymers was confirmed by FTIR Spectra, and ^{1}HNMR Spectra . The antimicrobial activity of the prepared polymers against different types of microorganisms including Gram-positive bacteria

(*Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa; Escherichia coli; Klebsiella pneumoniae ;* and *Salmonella typhi)* as well as fungi (*Alternaria solani, Fusarium oxeyspurum , Aspergillus niger ,* and *Mucor)*and yeast such as (*Candida albicans, , candida krusei , candida parapsilosis ,* and *candida tropicalis*).

The antimicrobial activity of polymers containing phenolic, Nitro , Chloro ,Amine , Sulfonic,and Carbonyl group , have high activity against Gram-negative and positive bacteria as well as fungi and yeast [26].

Generally, it was found that the diameter of the inhibition zone varied according to the tested microorganism as well as the polymer microstructure. The inhibition zone diameter increased from Homo-polymer passed to Co- polymer due to the increase in the numbers, and types of funictional groups in the polymers, At the meantime, the inhibition zone diameter increased for prepared polymers due to the increase in space length between the function groups and polymer backbone [26].

4.1. Antibacterial activities

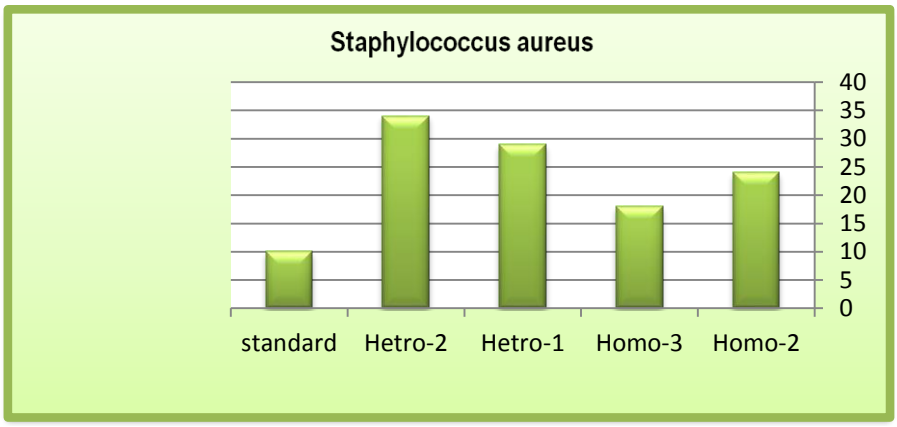
The antibacterial activity of prepared polymers have been studied by using Muller Hinton agar by inoculating 50 ml of fresh culture broth (18 hrs).

The prepared polymers against gram positive bacteria; *staphylococcus aureus* , gram negative bacteria; *Escherichia coli* , *Pseudomonas aeruginosa*, *Salmonella Typhi* and *Klebsiella pneumoniae* for 24 hr. at 37 ºC The inhibition zones were measured by using the disc method .The bacterial inhibition zone value are summarized in Table (4-1) and its statistical presentation is shown in following figures.

Table(4 -1):-Biological activity data for bacteria (zone of inhibition in mm) of Homo polymers and Co-polymers :

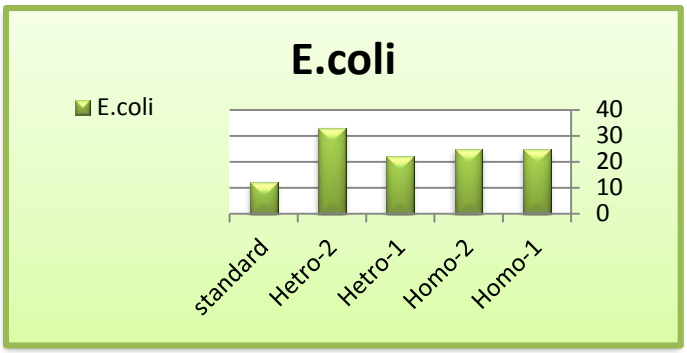
No.of polymers	Codes	zone of Inhibition				
		E.Coli	***Klebsiella pneumoniae***	***Pseudomon-as aeruginosa***	***Staphylococcus aureus***	***salmonella typhi***
Homo-1	PHAO**1**	25	25	15	33	19
Homo-2	PNAO1	25	26	17	24	14
Hetero-1	PHAO2	22	18	18	29	25
Hetero-2	PNAO2	33	25	24	34	28
standard	**Ciprofloxacin**	12	15	14	10	10

In the Homo polymers and co- polymers the results demonstrate that *staphylococcus aureus* were sensitive to all polymers , and its high sensitive to co-polymer 2 [$PNAO_2$] than co-polymer 1 [PHAO2] .



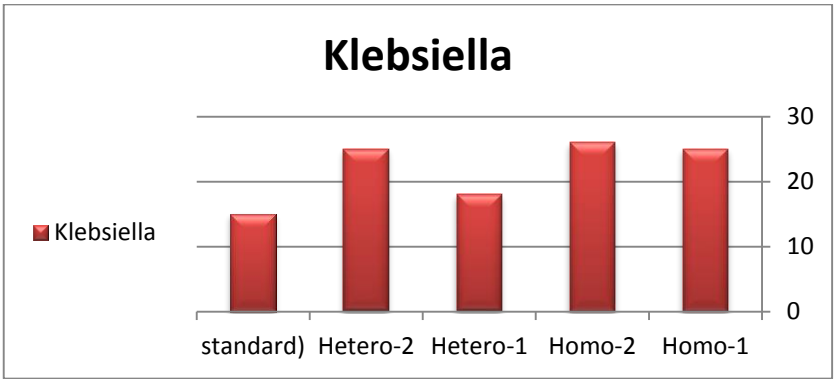
Figure(4-1):-Statistical representation for biological activity of prepared polymers on *Staphylococcus aureus* bacteria .

while the *Escherichia coli* show a good activity to co polymer 2, but give good sensitivety to Homo polymer 2, Homo polymer 1 and Co polymer 1 .



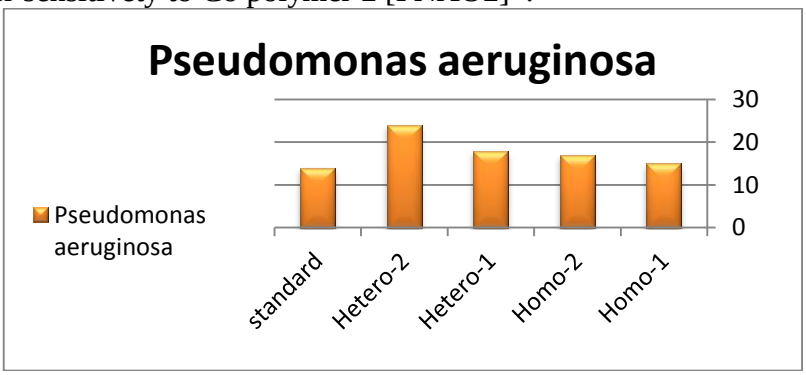
Figure(4-2):-Statistical representation for biological activity of prepared polymers on E.coli bacteria .

where the *Klebsiella pneumoniae* show a high sensitivety to Homo 2 [PNAO1] , but the other polymers have lower value than 28 mm to *Klebsiella pneumoniae* .



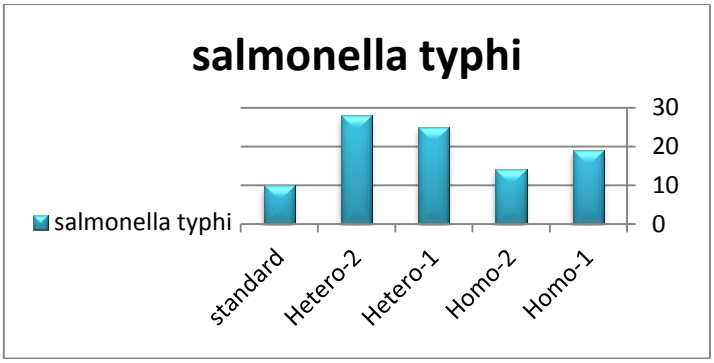
Figure(4-3):-Statistical representation for biological activity of prepared polymers on *Klebsiella pneumoniae* bacteria

The *Pseudomonas aeruginosa* , shows high sensitivity against all polymers , but given higher sensitivety to Co polymer 2 [PNAO2] .



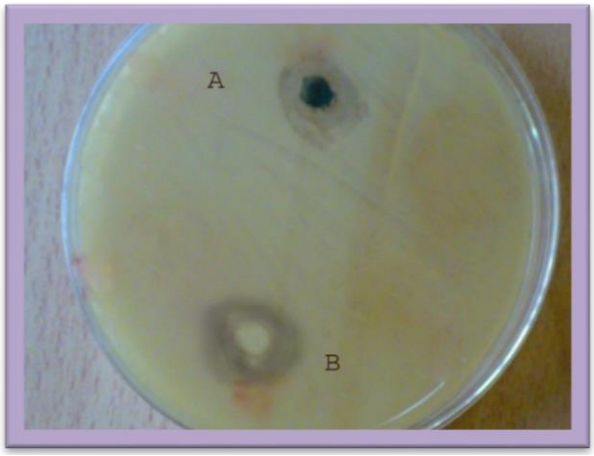
Figure(4-4):-Statistical representation for biological activity of prepared polymers on *Pseudomonas Areuginosa* bacteria .

The *Salmonella Typhi* , it was has high sensitivity to Co polymer 2 than other polymers .

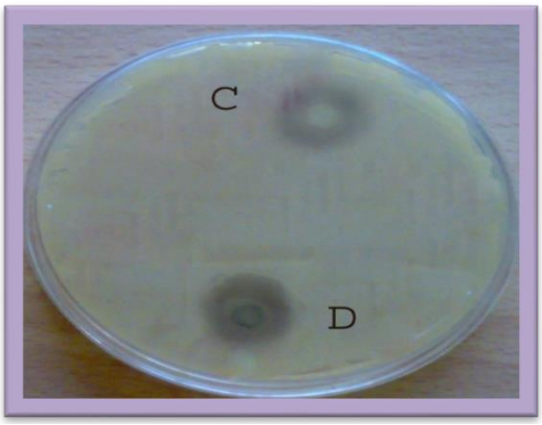


Figure(4-5):-Statistical representation for biological activity of prepared polymers on *Salmonella typhi* bacteria .

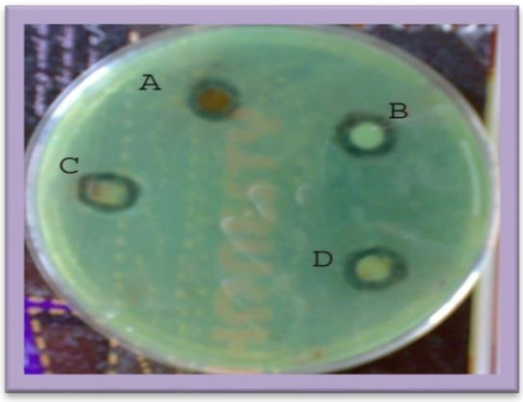
Finally all the prepared polymers give good sensitivity against all bacteria.
The following figures show the influence biological activity for prerared polymers concentration (500 ppm) with the bacteria under study, Three replicates were made for each test at 37°C for 24 hrs for bacterias. Then the average diameters of inhibition zones were recorded in millimeters (mm), and compared with standard antibacterial drug (ciprofloxacin).



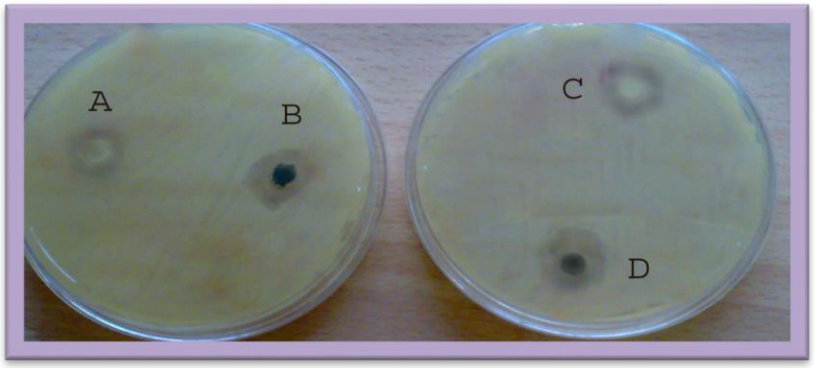
(A) Effect of (PHAO1) against *Klebsiella pneumoniae* bacteria
(B) Effect of (PNAO1) against *Klebsiella pneumoniae* bacteria.



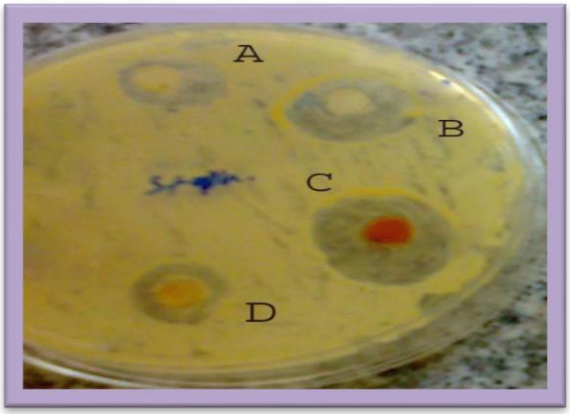
(C) Effect of (PHAO2) against *Klebsiella pneumoniae* bacteria
(D) Effect of (PNAO2) against *Klebsiella pneumoniae* bacteria



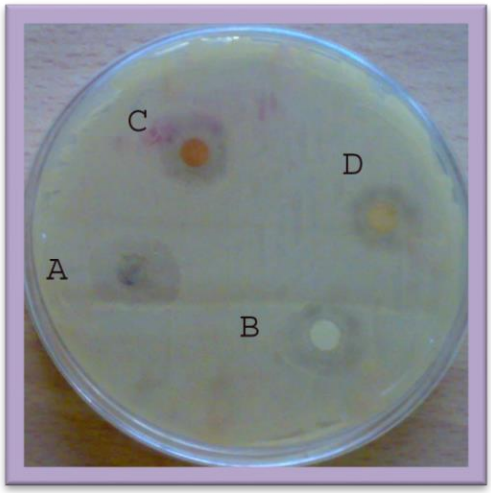
(A) Effect of (PHAO1) against *Pseudomonas Areuginosa* bacteria .
(B) Effect of (PNAO1) against *Pseudomonas Areuginosa* bacteria .
(C) Effect of (PHAO2) against *Pseudomonas Areuginosa* bacteria .
(D) Effect of (PNAO2) against *Pseudomonas Areuginosa* bacteria .



(A) Effect of (PHAO1) against E.coli bacteria .
(B) Effect of (PNAO1) against E.coli bacteria .
(C) Effect of (PHAO2) against E.coli bacteria .
(D) Effect of (PNAO2) against E.coli bacteria .



(A) Effect of (PHAO) against *Staphylococcus aureus* bacteria .
(B) Effect of (PNAO) against *Staphylococcus aureus* bacteria.
(C) Effect of ($PHAO_2$) against *Staphylococcus aureus* bacteria .
(D) Effect of ($PNAO_2$) against *Staphylococcus aureus* bacteria.



(A) Effect of (PHAO) against *Salmonella typhi* bacteria .
(B) Effect of (PNAO) against *Salmonella typhi* bacteria .
(C) Effect of ($PHAO_2$) against *Salmonella typhi* bacteria .
(D) Effect of ($PNAO_2$) against *Salmonella typhi* bacteria .

4.2- Antifungial activities

Afungus is a colorless plant like lacking chlorophyll. Fungi that cause disease in human may be yeast – like or mold – like and are called mycotic infections or fungal infections.Many fungi cause plant disease , but only about 100 of the thousand of known species of the yeast and molds cause disease in humans or animals Only the dermatophytes and candida are commonly transmitted from one human to another [27].

Mycotic infection may be one of two types :

1. Supercactial mycotic infections.
2. Deep (systemic) mycotic infections.

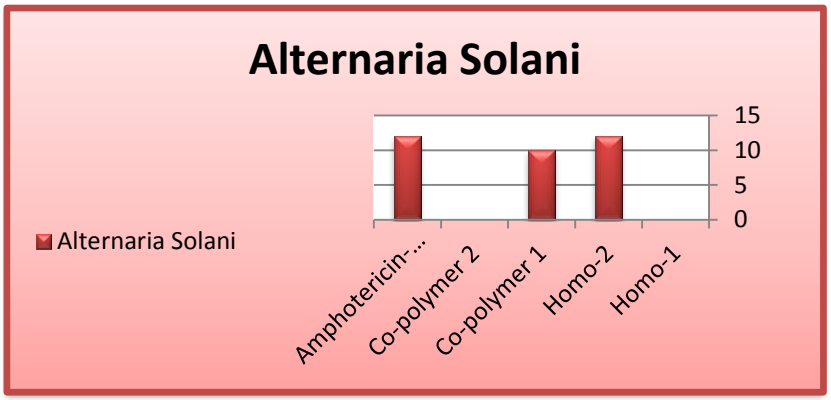
The superficial mycotic infection are those occurring inside the body , such as in the lungs. Treatment of deep mycotic infections is often difficult and prolonged . Antifungal drugs may be fungicidial or fungistatic. Antifungal drugs are used in the treatment of superfacial and deep fungal infections . The specific uses of the antifungal are given elsewhere Amphoteria B . is the most effective drug available for the treatment of most systemic fungal infection [28] . Fungal infection of the skin or muscous membrane may be treated with topical or vaginal preparation.

The antifungal activity of prepared polymers have been studied by using the pure cultures of pathogenic fungi viz. *Alternaria solani*, *Fusarium oxeyspurum* , *Aspergillus niger* , and *Mucor)* were used for antifungal activit studies. Antifungal activity was evaluated using poisoned food technique on potato dextrose agar (PDA) medium.The pure cultures of microorganism (7 days old culture at 37 ºC and inhibition zones were measured in melli meter.

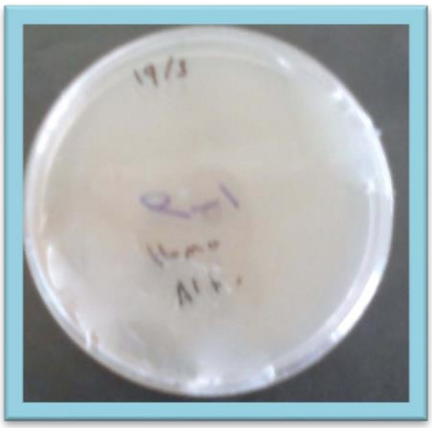
Table 4-2 : Biological activity data (zone of inhibition in mm) of Homo and co – polymers aginest fungal pathogens :

No.of polymers	codes	zone of Inhibition			
		Alternaria Solani	*Fusarium Osey Sporum*	*Mucor*	*Aspergillus niger*
Homo-1	PHAO1	**-**	**-**	**-**	**-**
Homo-2	PNAO1	**12**	**14**	**10**	**18**
Co-polymer 1	PHAO2	**10**	**15**	**11**	**32**
Co-polymer 2	PNAO2	**-**	**-**	**-**	**-**
Standard	**Amphotericin -B**	**12**	**6**	**6**	**9**

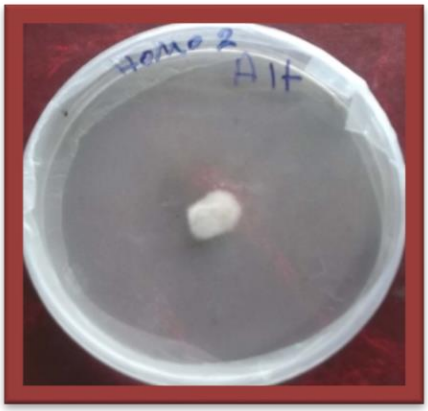
signal (-)mean the growth of microorganism equal to Zero , 100% kill percintg . The result demonstrate that The (PHAO1 ,PNAO2) rise to 100% perecintage for the *Alternaria Solani* fungi,and other polymers give good sinsitivity vary from (10 mm) in (PHAO2) to be greater (12 mm) in co –polymer 8 (PNAO1).



Figure(4-6):-Statistical representation for biological activity of prepared polymers on *Alternaria Solani* .

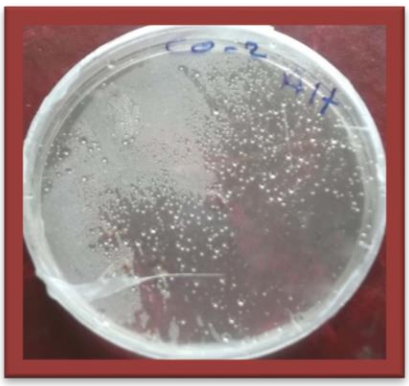


(A)



(B)

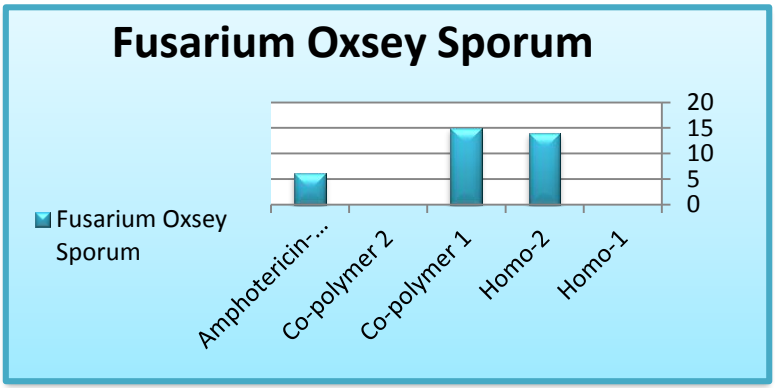
(C)



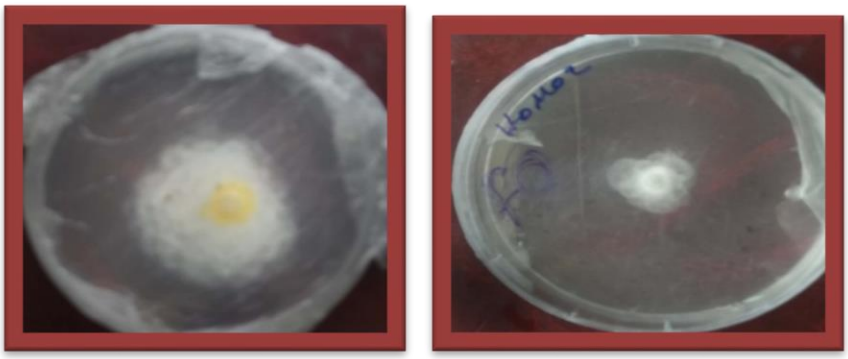
(D)

(A) Effect of (PHAO1) against *Alternaria Solani* .
(B) Effect of (PNAO1) against *Alternaria Solani* .
(C) Effect of (PHAO2) against *Alternaria Solani* .
(D) Effect of (PNAO2) against *Alternaria Solani* .

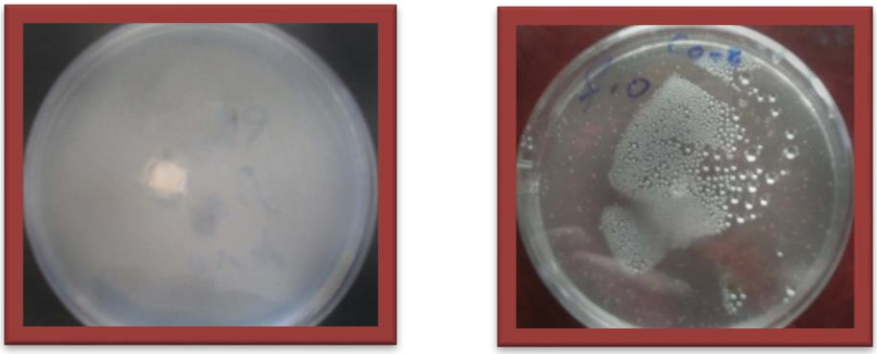
Despite that Fusarium oxseysporum shows less activity agenist (PHAO2) ,but it gives growth equal to zero against (PHAO1 , PNAO2) while the result was (14 mm) in (PNAO1).



Figure(4-7):-Statistical representation for biological activity of prepared polymers on *Fusarium Oxsey Sporum* .



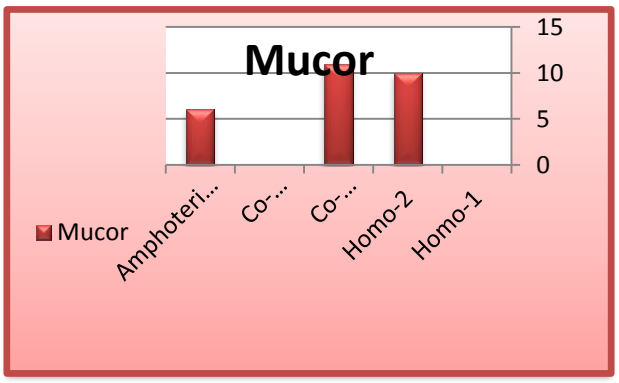
(A) (B)



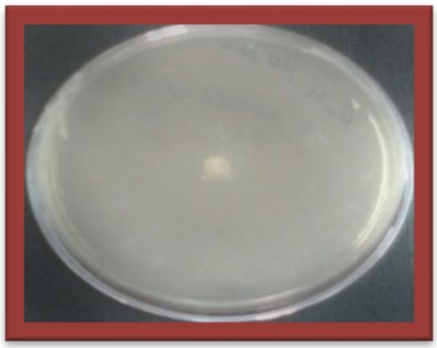
(C) (D)

(A) Effect of (PHAO1) against *Fusarium oxeyspurum* .
(B) Effect of (PNAO1) against *Fusarium oxeyspurum* .
(C) Effect of (PHAO2) against *Fusarium oxeyspurum* .
(D) Effect of (PNAO2) against *Fusarium oxeyspurum* .

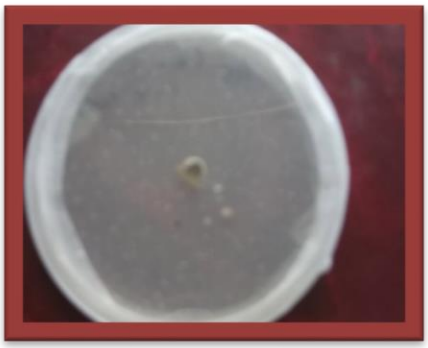
The Mucor fungi when tested to determine the antifungal activites gives the largest result aginst polymers (PHAO1 , PNAO2) reaching 100% percentage to kill pathogens on solidification media ,while the less effect to be (11 mm) in (PHAO2).



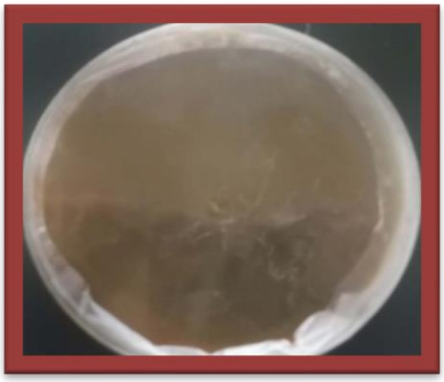
Figure(4-8):-Statistical representation for biological activity ofprepared polymers on *Mucor*



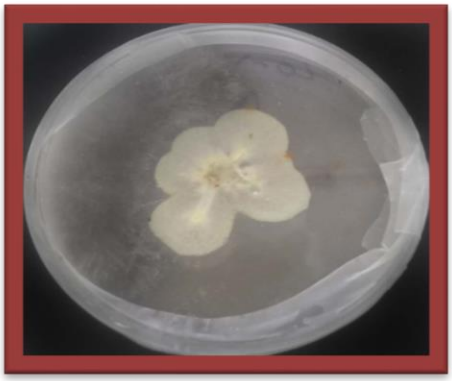
(A)



(B)



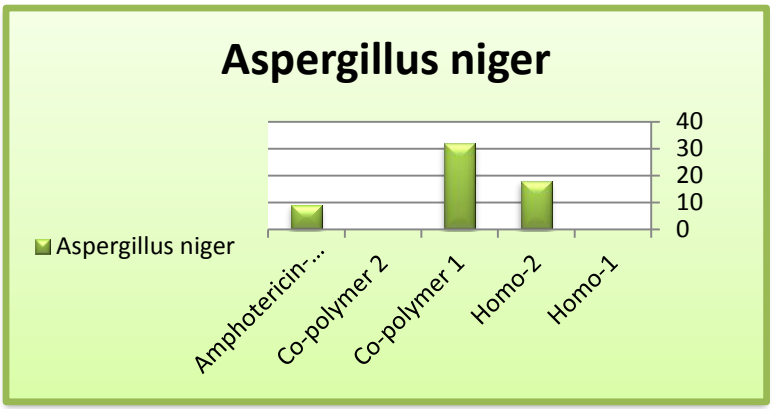
(C)



(D)

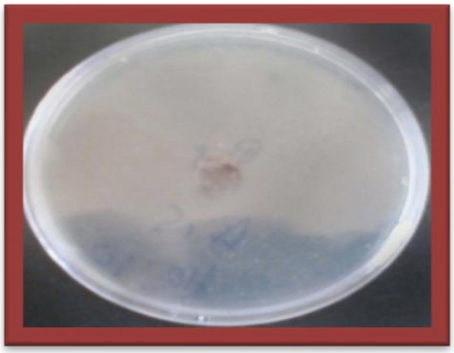
(A) Effect of (PHAO1) against *Mucor*.
(B) Effect of (PNAO1) against *Mucor*.
(C) Effect of (PHAO2) against *Mucor*.
(D) Effect of (PNAO2) against *Mucor*.

When tested the prepared polymers on Aspergillus niger Fungi we get the higher effect by the polymers (PHAO1, and PNAO2) gives zero mm growth in pathogens , but in polymer (PHAO2) gives result equal to (32 mm) which can consider the lowest effect on fungi .

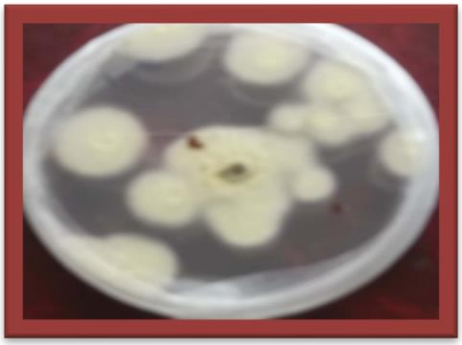


Figure(4-1):-Statistical representation for biological activity of prepared polymers on *Aspergillus niger*

(A)



(B)



(C)

(D)

(A) Effect of (PHAO1) against *Aspergillus niger*
(B) Effect of (PNAO1) against *Aspergillus niger* .
(C) Effect of (PHAO2) against *Aspergillus niger* .
(D) Effect of (PNAO2) against *Aspergillus niger* .

4.3.1 Anti yeast assay for newly synthesized polymers

Polymeric antimicrobial agents represent a new and important direction that is developing in the field of antimicrobial agents. The treatment of fungal infections in general accentuates a real problem due to the limited effectiveness for the available antifungal agents and its severe side effects. The current disturbance in the immune system and the global HIV pandemic have resulted in amassive increase in the incidence of systemic fungal infections. The therapy of deep yeast infections, particularly those caused by opportunistic pathogens, such as *Candida albicans*, remains a difficult medical problem [28]. The antifungal agents that are still most frequently used, are those that affects the fungal cytoplasmic membranes [29]. The antifungal treatments need lifelong therapy, because of its toxicity and high cost of their production. So, we need a new series of antifungal compounds that have a high efficiency and low cost. Therefore, we have started to test different low molecular weight antimicrobial agents ,to prevent the residual toxicity of these low molecular weight agents and to increase the lifetime of the agents, polymeric antimicrobial agents were developed recently ,water insoluble polymers with antimicrobial activity have many biomedical applications. They are used in the production of medical fibers, ears socks and package materials [30].

4.3.2.Anti Yeast Activity

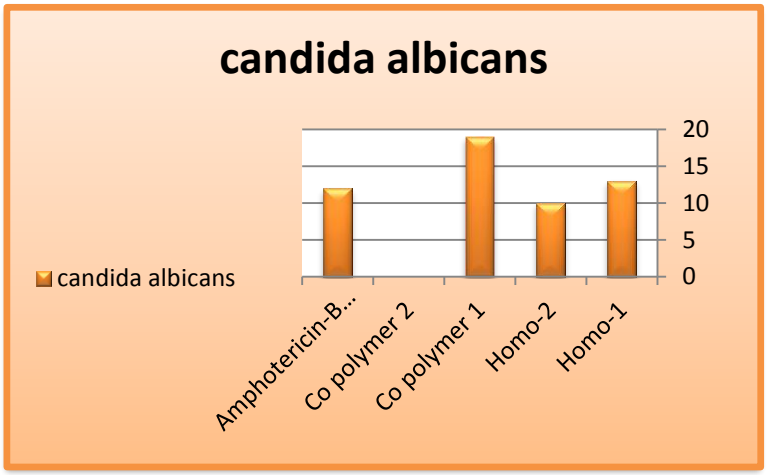
Many antifungal agents have been discovered, but only a few are active both *in vivo* and *in vitro*. Therefore, we have planned to look for new classes of newly synthesized chemical polymers. Two of the different
screened newly synthesized polymers have proved to be active against tested *Candid* . Due to the insolubility or the poor solubility of these polymers, they have been tested by the poisioned food technique on potato dextrose agar (PDA) medium .the pure cultures of microorganism (7 days old culture at 37^{o}C , and three replicates were made for each test, then the average diameters of inhibition zones were recorded in millimeters (mm) and compared with standard antifungal drug (Amphoteria B) [31].
The following table (4-3) show the results for the prepared polymers were tested with Candida types.

Table(4-3) Biological activity data (zone of inhibition in mm) of Homo polymers and Co-polymers against Yeast :

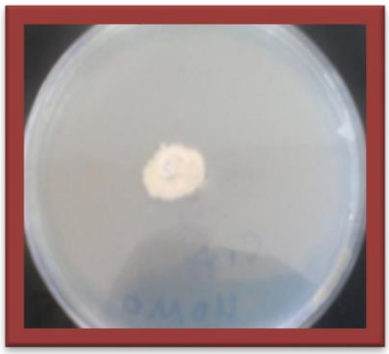
No.of polymers	codes	zone of Inhibition			
		candida albicans	*candida krusei*	*candida parapsilosis*	*candida tropicalis*
Homo-1	PHAO1	**13**	**17**	**-**	**-**
Homo-2	PNAO1	**10**	**7**	**18**	**-**
Co-polymer 1	PHAO2	**19**	**19**	**16**	**-**
Co-polymer 2	PNAO2	**-**	**-**	**-**	**-**
Standard	**Amphotericin -B**	**12**	**6**	**6**	**9**

signal (-)mean the growth of microorganism equal to Zero , 100% kill percintg .

Candida albicans was largely affected by polymer (PNAO2), to give the 100% percentage for killing the candida albicans, while the lowest result to be (19mm) in (PHAO2).

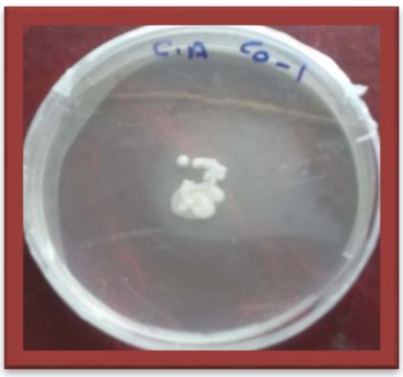


Figure(4-10):-Statistical representation for biological activity of prepared polymers on*Candida albicans* .



(A)

(B)

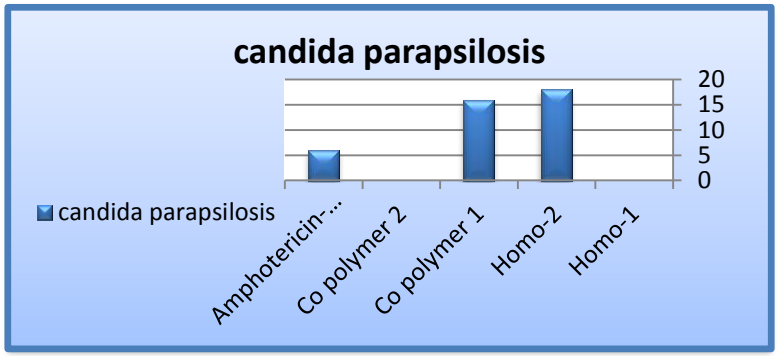


(C)

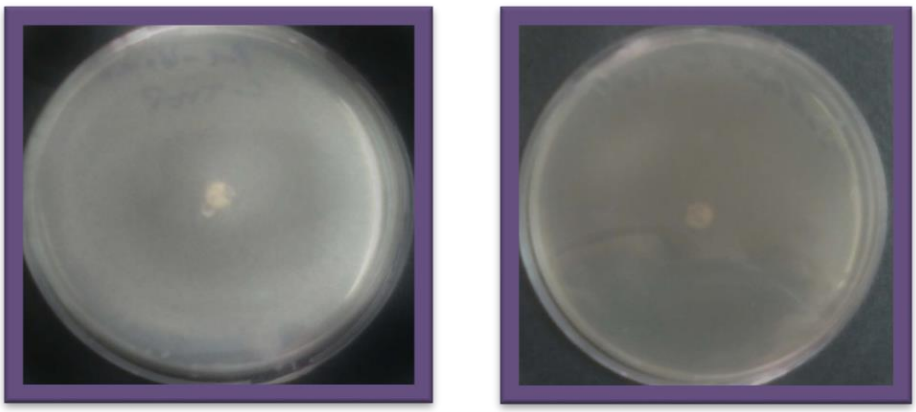
(D)

(A) Effect of (PHAO1) against *candida albicans* .
(B) Effect of (PNAO1) against *candida albicans* .
(C) Effect of (PHAO 2) against *candida albicans* .
(D) Effect of (PNAO 2) against *candida albicans* .

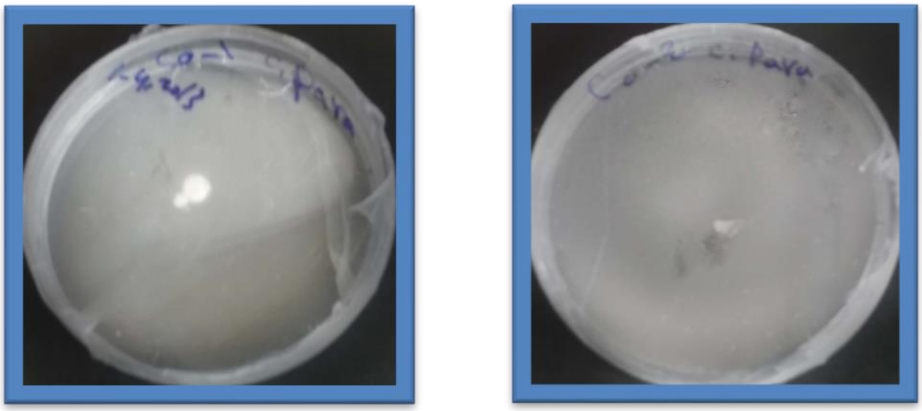
While the Candida parapsilosis shows high sensitivity to (PHAO1 , PNAO2) that give sensitivity equal to 100% percentage , while the lowest result to be (18mm) in (PNAO1).



Figure(4-11):-Statistical representation for biological activity of prepared polymers on *Candida Parapsilosis*



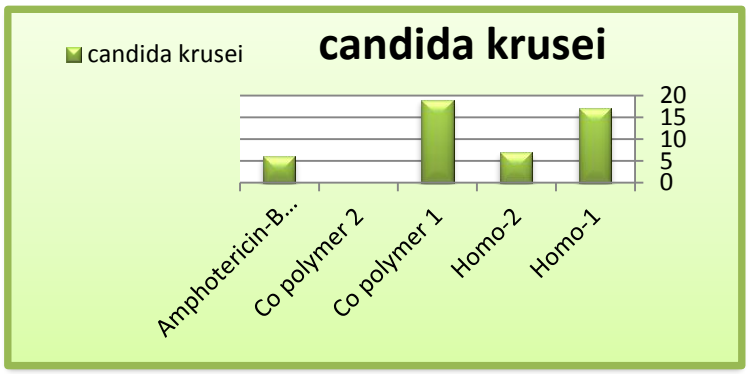
(A) **(B)**



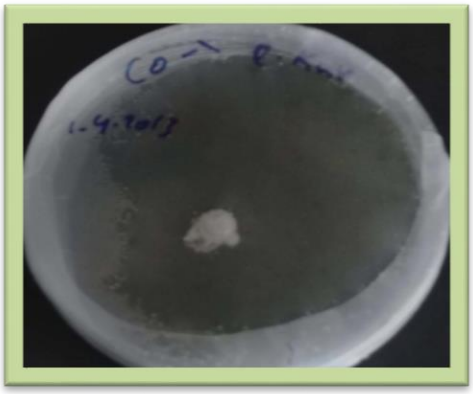
(C) **(D)**

(A) Effect of (PHAO1) against *candida parapsilosis*.
(B) Effect of (PNAO2) against *candida parapsilosis*.
(C) Effect of (PHAO2) against *candida parapsilosis*.
(D) Effect of (PNAO2) against *candida parapsilosis*.

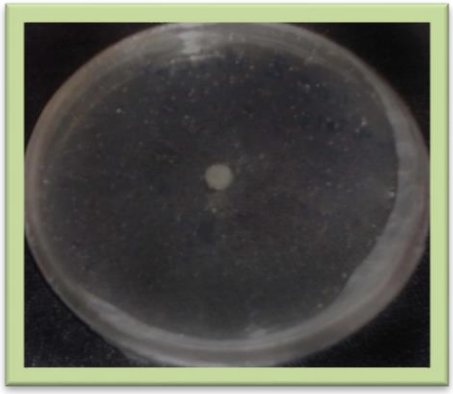
where *Candida krusei showed* good effected to (PNAO2) polymer the kill percentage rise to (100%) ,but remains polymers give results variable from lowest value (7mm) in (PNAO1) polymer to rise (19 mm) in (PHAO2) polymer .



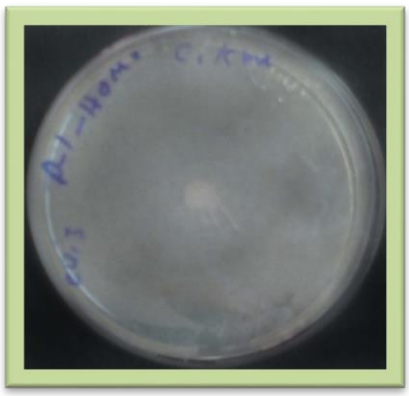
Figure(4-12):-Statistical representation for biological activity of prepared polymers on *Candida Krusei.*



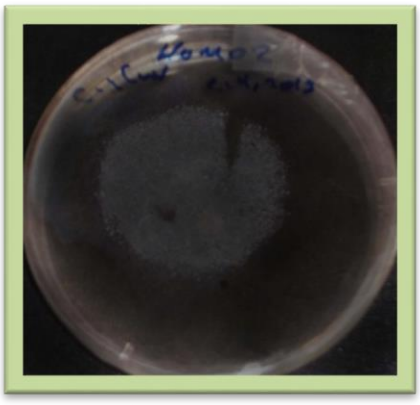
(A)



(B)



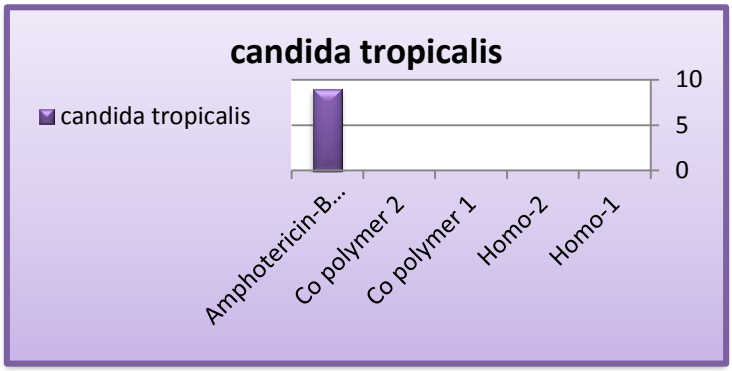
(C)



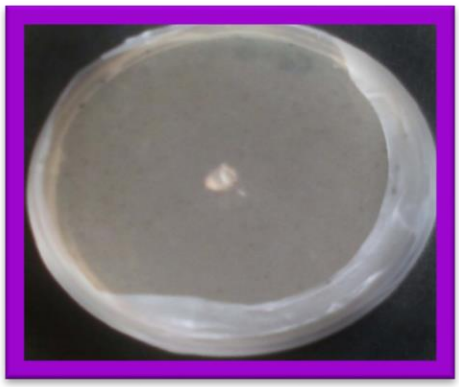
(D)

(A) Effect of (PHAO1) against *candida krusei* .
(B) Effect of (PNAO1) against *candida krusei* .
(C) Effect of (PHAO2) against *candida krusei* .
(D) Effect of (PNAO2) against *candida krusei* .

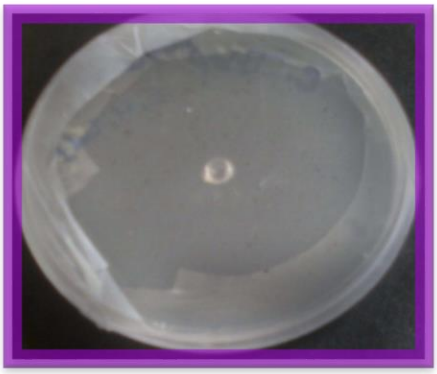
The last type from Candida which used in this study are Candida Tropicalis ,when test this type give very good sensitivity to all polymers such as (PHAO1, PNAO1, PHAO2 ,PNAO2) while give zero growth with kill percentage equal to 100% .



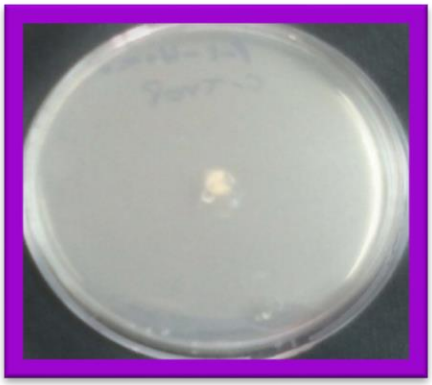
Figure(4-13):-Statistical representation for biological activity of prepared polymers on *Candida Tropicalis.*



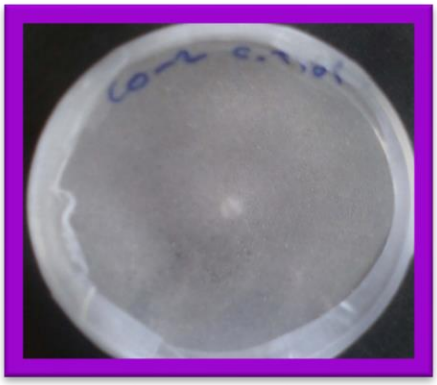
(A)



(B)



(C)



(D)

(A) Effect of (PHAO1) against *candida tropicalis* .
(B) Effect of (PNAO1) against *candida tropicalis* .
(C)Effect of (PHAO2) against *candida tropicalis* .
(D) Effect of (PNAO2) against *candida tropicalis* .

Finally all polymers given good sensitivity against all yeast but largest effected was shown by (PNAO2) polymers while all type of Candida was given zero growth on solidification media when treated with Candida pathogens.

The purpose of this study was to find a new pharmaceutical compound to inhibit the growth of pathogenic microorganisms. Two types of the polymers, Homo polymer and Co-polymers ,were selected for their activity against *C. albicans, C. krusi , C.parapsilosis,* which was involved in systemic fungal infections and *C. tropicalis* which emerged as an important pathogen in neutropenic cancer patients [31].

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*تخليق وتشخيص أنواع جديدة من البوليمرات المضادة للمايكروبات ودراسة فعاليتها البايولوجية

تاريخ الاستلام : 2013\11\7 تاريخ القبول : 2014\1\27

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الخلاصة :

في هذا البحث تم تخليق بوليمرات جديدة تتضمن بوليمرات متجانسة ويعتمد التخليق على وحدات مركب الأوكزيم Hydroxylamine كوحدات اولية التي يمكن دمجها مع P-Hydroxyacetophenone,P-Nitroacetophenoneمع في وجود NaOH والبوليمرات المتجانسة تم تصنيعها من خلال تفاعل الأوكسيم مع بعضها البعض والفورمالديهايد في وجود حامض الهيدروكلوريك كعامل مساعد ، بينما تم تحضير البوليمرات الغير متجانسة من تفاعل مركب الأوكسيم مع مونومرات أخرى مثل (Toluensulphonic acid , 2,4-Dichlorobenzoic acid) والفورمالديهايد في وجود حامض الهيدروكلوريك كعامل مساعد ، أختبرت البوليمرات المحضرة كمضادات للبكتريا ، وكمضادات للفطريات وكذلك مضادات للخمائر بواسطة دراسة تأثيرها على العديد من الاحياء المجهرية المتضمنه البكتريا والخمائر والفطريات ،كل البوليمرات المحضرة اظهرت فعالية بايولوجية عالية جدا مقارنة مع ciprofloxacin and amphotericin –B كأدوية قياسية .

الكلمات المفتاحية :بوليمرات مضادة للاحياء المجهرية ، مضادات الاحياء المجهرية ، بوليمرات .

Chemistry classification : QD 241- 441

***The Research is apart of on M.Sc. thesis in the case of the Second researcher**