Synthesis and Characterization New Type Antimicrobial Polymers and Study Biological Activity

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Rafid K. Kmal
Department of Chemistry, College of Education, University of Al-Qadisyia
rafid_qais@yahoo.com

Abstract :

This research describes the synthesis new co-polymers which can synthesized from the reaction of (Toluen sulphonic acid , 2,4-Di chloro benzoic acid , p-hydroxy benzoic acid, Aniline, and p-Bromo phenol) with (Hydroquinone, chloro benzoic acid, 5-Sulphosalicalic acid,and p-Bromo aniline) respectively and formaldehyde in presence of HCl as catalyst, These polymers were identified by FT-IR 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.

Keywords: *Antimicrobial Polymers*, *Biological Activity*, synthesis new co-polymers

Chemistry classification : QD241 - 441

1. Introduction:

Antimicrobial polymers are commonly obtained either by synthesizing monomeric biocide moieties and then polymerizing subsequently or copolymerizing with another monomer [9]. The grafting of antimicrobial agents into natural occurring or synthetic polymers is also another approach to obtain such materials in various applications [10,11,38-39].

Antimicrobial polymers are commonly obtained either by synthesizing monomeric biocide moieties and then polymerizing subsequently or copolymerizing with another monomer [11-16].

Microbial infection constitutes one of the most serious problems in several areas in which hygiene holds a great importance (*e.g.* medical devices, drugs, health care applications, water purification systems, textile, and food packaging). Antimicrobial agents are materials which are capable of killing such pathogenic microorganisms [1]. Low molecular weight antimicrobial agents

have been being widely used for the sterilization of water, as antimicrobial drugs (antibiotics), as food preservatives, and for soil sterilization [2]. However, these agents have many disadvantages, such as short-term antimicrobial ability and toxicity to the environment [3-5]. In the last two decades, novel antimicrobial polymers have offered a promise for antimicrobial applications by minimizing the environmental problems (by reducing the residual toxicity of the agents) and prolonging the lifetime of the antimicrobial agents. Also, polymeric antimicrobial agents are nonvolatile, chemically stable, do not permeate through skin and have been observed to reduce the incidences of the infection caused biomaterial implant failures [6-8].

The mechanism of action of antimicrobial systems is based on either the slow release of toxic antibacterial agents or contact killing (no release of any active agent) [17,18]. Tethering long-tailed cationic moieties to polymer matrixes, as an example to the latter case, has been studied extensively by various research groups [19,20-24]. Especially, Klibanov *et al.* have been done comprehensive studies on contact killing polymeric materials [24-27,30,23]. It is well-known that immobilized quaternary ammonium compounds (if they have sufficiently long hydrophobic tails) possess antibacterial properties by interacting with and disrupting bacterial cell membranes [28,38]. When the bacterial cells are in a contact with such surfaces, long hydrophobic chains presumably penetrate and disrupt the cell membranes. One major drawback of such commonly used systems is their unnecessary antimicrobial compound concentration in the bulk [31] .Our approach is to use surface segregation of these compounds since only the ones at the top of the surface are biocidal [29].

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 [32]. In this research is to prepared the new co-polymers as antimicrobial polymers and test against different types of microorganisms for determination the biological activity including as Gram-positive bacteria *Staphylococcus aureus*, and Gram-negative bacteria *Pseudomonas aeruginosa; Escherichia coli ,Klebsiella pneumoniae ,* and *Salmonella typhi.* Then test the effect the prepared polymers against Fungi pathogens such as *Alternaria solani*, *Fusarium oxeyspurum* , *Aspergillus niger* , and *Mucor.*Finally, the syntheses polymers examined againstYeast pathogens such as *Candida albicans*, , *candida krusei* , *candida parapsilosis* , and *candida tropicalis* . This 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

P- Nitro benzoicacid (MERCK) , P - Nitroacetophenone(ALDRICH)., P -Hydroxy benzoic acid (ALDRICH)., NaOH (BDH) , Formaldehyde (ALDRICH). , HCl (HIMEDA), ,Hydroquinone (MERCK), Aniline, (HIMEDA) , 5-Sulphosalicalic acid(MERCK), p-Bromo phenol (MERCK) , p-Bromo aniline (BDH) .

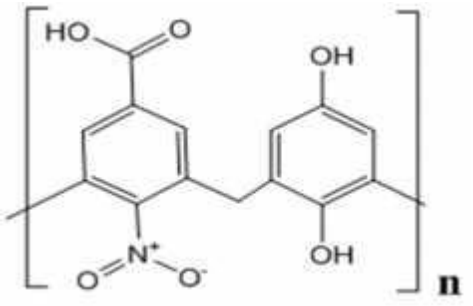
2-2 *.Apparatus*

(FTIR 8400S) Forier Transform infrared spectrophotometer,Shimadzu,Jaban,(Hot plate stir) Bibby Strlintd .

2.3 Methods

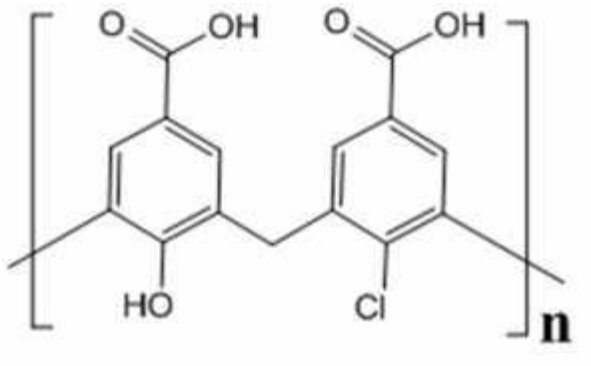
2.3.1 Co- Polymer Synthesis

A mixture of P- Nitro benzoicacid (0.01 mol), condensing reagent formaldehyde (0.02mol) and the co-monomer Hydroquinone (0.1 mol),(0.88 gm) 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 [33].

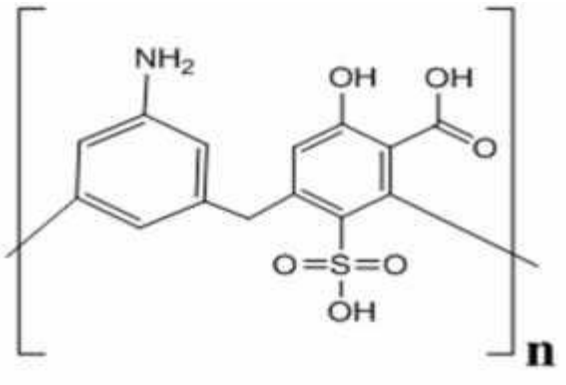


Scheme 2 -1 Structure Of (PHNBA)

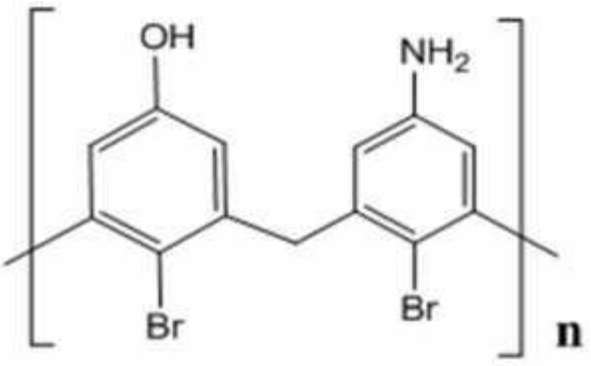
The other polymers were prepared by the same as above procedure .



Scheme 2 -2 Structure Of (PHBAC)



Scheme 2 -3 Structure Of (PANSA)



Scheme 2 -4 Structure Of (PBPA)

Table2.1 represents the color, yield and melting point for preparing of Co polymers :

NO	Polymers	Substance		Color	Yield%	M.P/ c
1	Co-polymer 1	P- Nitro benzoicacid (1.66 g) (0.0099 mol)	Hydroquinone (0.88g)(0.044 mol)	White	90	125
2	Co-polymer2	p-hydroxy benzoic acid (1.66 g)(0.012 mol)	chloro benzoic acid (0.88g)(0.036 mol)	Yellow	70	165
3	Co-polymer3	Aniline(1.66g) (0.017mol)	5-Sulphosalicalic acid (0.88 g)(0.042 mol)	Orang	81	134
4	Co-polymer4	p-Bromo phenol (1.66g)(0.0095mol)	p-Bromo aniline (0.88 g)(0.044 mol)	White	95	75

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, six wells (five in periphery and one in centre) 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 37° 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 [33].

2.5. Antifungal and Yeast activities

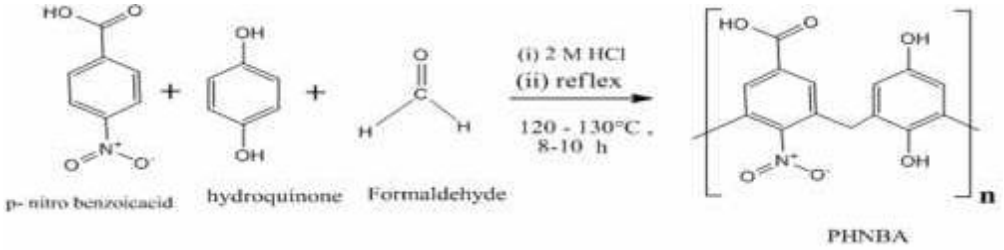
Pure cultures of pathogenic fungi *viz.*(*Alternaria solani*, *Fusarium oxeyspurum* , *Aspergillus niger* , *and Mucor*) *and the Yeast is* (*candida albicans* , *candida krusei* , *candida parapsilosis* , *and candida tropicalis)* were used for antifungal activity studies. Antifungal activity of the 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 [33].

Results & Dissections

3.1. ***Synthesis and Characterization of CO-Polymers:***

3.3.1 ***Synthesis and Characterization of (PHNBA):***

The (PHNBA) was synthesized from the reaction of P- Nitro benzoicacid with Hydroquinone and formaldehyde in presence of HCl as catalyst by refluxing for 8 – 10 hrs. this reaction was shown in Scheme (3.1).[33]



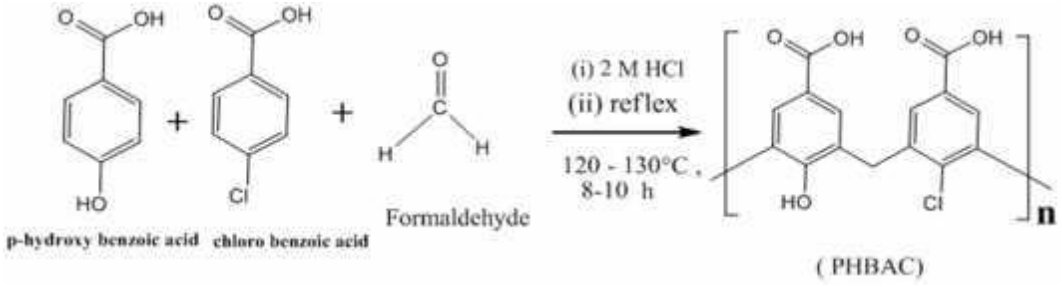
Scheme 3.1

In Figure (1.3) The FTIR spectra of (PHNBA) shows the absorption band of C=C Aromatic at 1660 cm^{-1}, C-H Aromatic at 3160 cm^{-1}, OH at 3100 cm^{-1}, N=O at 1350 cm^{-1}, 1545 cm^{-1}, , N-O at 930 cm^{-1} and C-OH at 3390 cm^{-1}.

Figure (3.1) The FTIR spectra of (PHNBA)

3.3.2 Synthesis and Characterization of (PHBAC):

The (PHBAC) was synthesized from the reaction of p-hydroxy benzoic acid with chloro benzoic acid and formaldehyde in presence of HCl as catalyst by refluxing for 8 – 10 hrs. this reaction was shown in Scheme (3.2).[33]



Scheme 3.2

In Figure (3.2)The FTIR spectra of (PHBAC) shows the absorption band of phenol 3660 cm^{-1}, OH- carboxylic acid at 3450 cm^{-1} ,,Cl –C at 780 cm^{-1} , CH Aliphati at 2980 cm^{-1} ,and C=C at 1690 cm^{-1}.

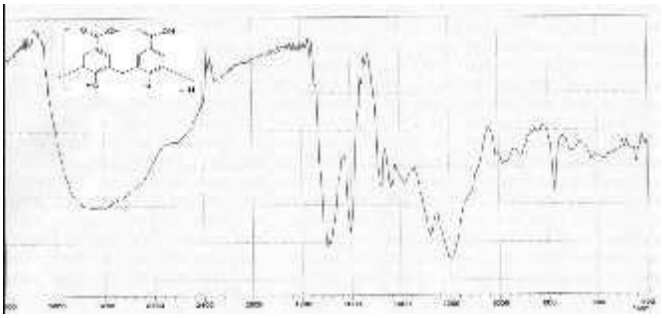
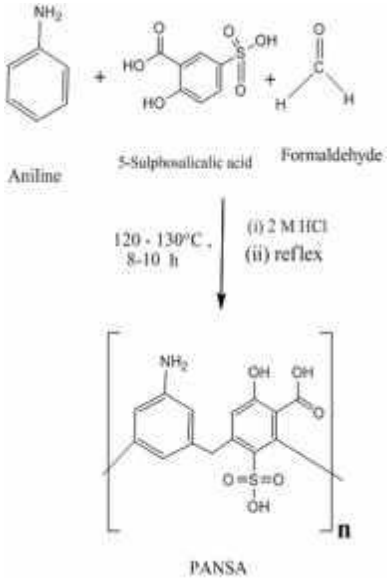


Figure (3.2) The FTIR spectra of (PHBAC)

3.3.3 Synthesis and Characterization of (PANSA):

The (PANSA) was synthesized from the reaction of Aniline with 5-Sulphosalicalic acid and formaldehyde in presence of HCl as catalyst by refluxing for 8 – 10 hrs. this reaction was shown in Scheme (3.3).[33]



Scheme 3.3

In Figure (3.3) The FTIR spectra of (PANSA) shows the absorption band of C=C Aromatic at 1680 cm^{-1}, C-H Aromatic at 3100 cm^{-1}, OH at 3100 cm^{-1},S=O at 1370 cm^{-1}, C=N at 1340 cm^{-1} , N-H at 3150 cm^{-1} and C-N at 1340 cm^{-1}.

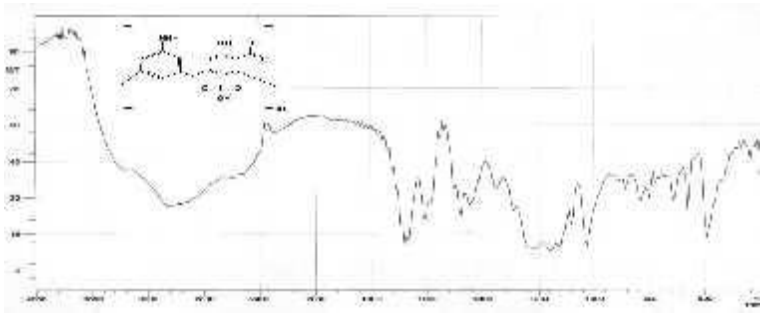
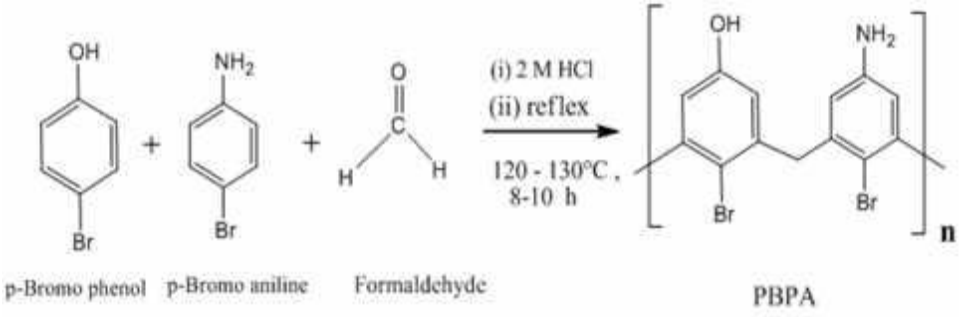


Figure (3.3) The FTIR spectra of (PANSA)

3.3.4 Synthesis and Characterization of (PBPA):

The (PBPA) was synthesized from the reaction of Aniline with 5-Sulphosalicalic acid and formaldehyde in presence of HCl as catalyst by refluxing for 8 – 10 hrs. this reaction was shown in Scheme (3.4).[33]



Scheme 3.4

In Figure (3.4)The FTIR spectra of (PBPA)shows the absorption band of phenol 3650 cm^{-1}, C-N at 1300 cm^{-1},NH2 at 3220 cm^{-1},Br-C at 590 cm^{-1} , and C=C at 1690 cm^{-1}.

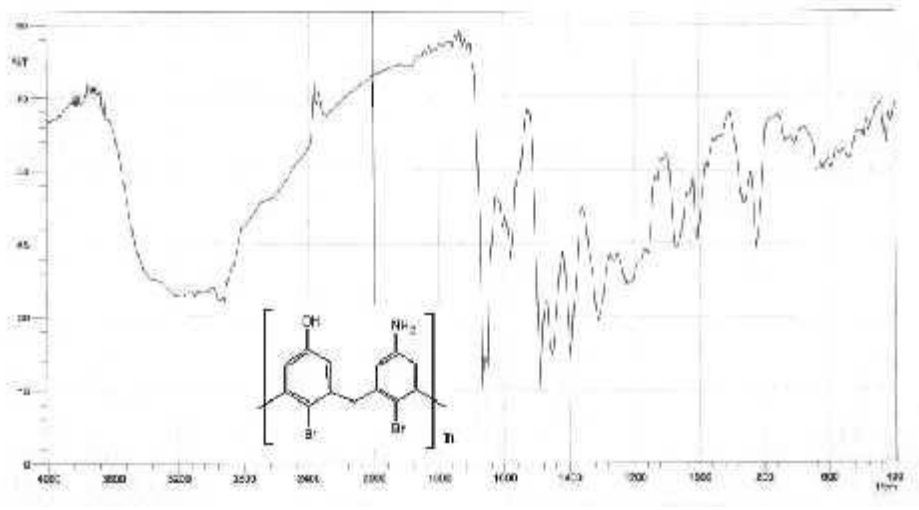


Figure (3.4) The FTIR spectra of (PBPA)

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]

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-Qadisiyia University, College of Science Biology Department .

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. 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 same time, 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

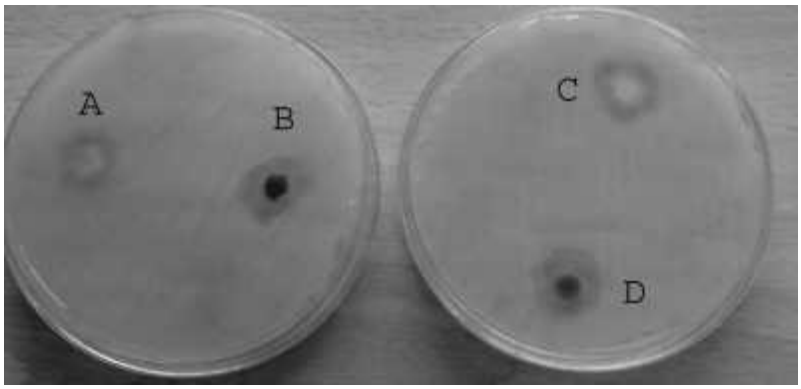
summarized in Table (4-1) and its statistical presentation is shown in following figures.

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*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*salmonella typhi*
Co-polymer 1	PHNBA	12	6	12	21	20
Co-polymer 2	PHBAC	8	19	5	15	16
Co-polymer 3	PANSA	15	16	20	8	14
Co-polymer 4	PBPA	9	14	12	11	13
Standard	Ciprofloxacin	12	15	14	10	10

The 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).

In the Homo polymers and co- polymers the results demonstrate that *E.coli* were sensitive to all polymers , and its high sensitive to co-polymer 3 [PANSA] than co-polymer 2 [PHBAC] .



Figure(3.5):-Statistical representation for biological activity of prepared polymers on *E.Coli* bacteria .

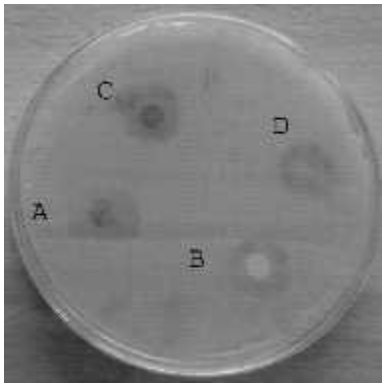
(A) Effect of (PHNBA) against *E.Coli* bacteria

(B) Effect of (PHBAC) against *E.Coli* bacteria.

(C) Effect of (PANSA) against *E.Coli* bacteria

(D) Effect of (PBPA) against *E.Coli* bacteria.

where the *Klebsiella pneumoniae* show a high polymers have lower value than 19 mm to *Klebsiella* sensitivety to Co polymer 2 [PHBAC] , but the other *pneumoniae* .



Figure(3.6):-Statistical representation for biological activity of prepared polymers on *Klebsiella pneumoniae* bacteria.

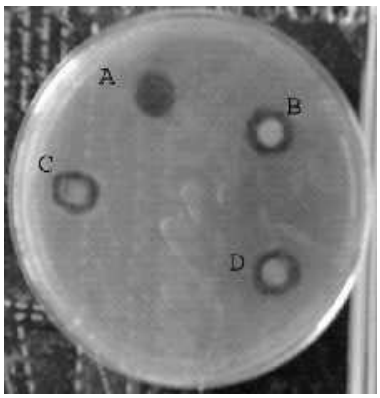
(A) Effect of (PHNBA) against *Klebsiella pneumoniae* bacteria

(B) Effect of (PHBAC) against *Klebsiella pneumoniae* bacteria.

(C) Effect of (PANSA) against *Klebsiella pneumoniae* bacteria

(D) Effect of (PBPA) against *Klebsiella pneumoniae* bacteria.

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



Figure(3.7):-Statistical representation for biological activity of prepared polymers on *Pseudomonas Areuginosa* bacteria .

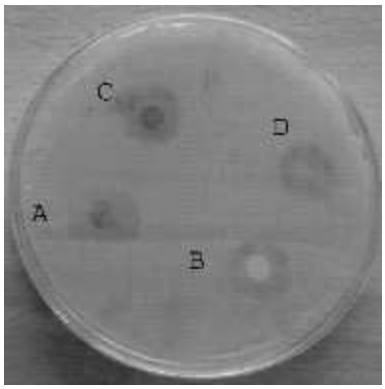
(A) Effect of (PHNBA) against *Pseudomonas Areuginosa* bacteria

(B) Effect of (PHBAC) against *Pseudomonas Areuginosa* bacteria.

(C) Effect of (PANSA) against *Pseudomonas Areuginosa* bacteria

(D) Effect of (PBPA) against *Pseudomonas Areuginosa* bacteria.

The *Salmonella Typhi* , it was has high sensitivity to Co polymer 1 (PHNBA) than other polymers .



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

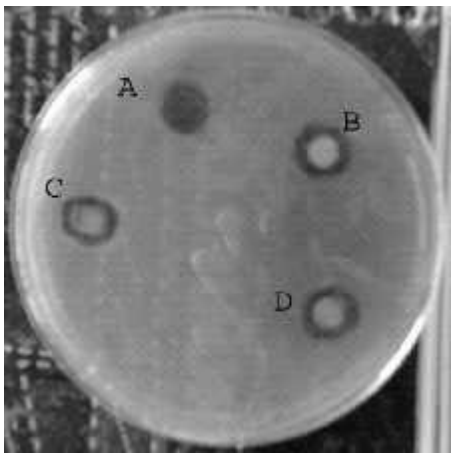
(A) Effect of (PHNBA) against *Salmonella typhi* bacteria

(B) Effect of (PHBAC) against *Salmonella typhi* bacteria.

(C) Effect of (PANSA) against *Salmonella typhi* bacteria

(D) Effect of (PBPA) against *Salmonella typhi* bacteria.

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



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

(A) Effect of (PHNBA) against *Staphylococcus aureus* bacteria

(B) Effect of (PHBAC) against *Staphylococcus aureus* bacteria.

(C) Effect of (PANSA) against *Staphylococcus aureus* bacteria

(D) Effect of (PBPA) against *Staphylococcus aureus* bacteria.

Finally all the prepared polymers give good sensitivity against all 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.

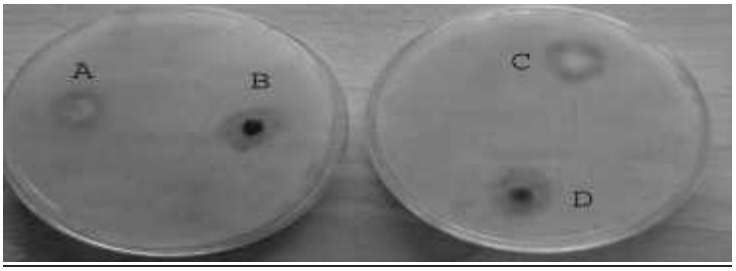
The antifungal activity of prepared polymers have been studied by using the pure cultures of pathogenic fungi viz. *Alternaria solani*, *Fusarium oxeyspurum* , *Aspergillus niger* , and *Mucor)* were used for antifungal activit studies. Antifungal activity

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.

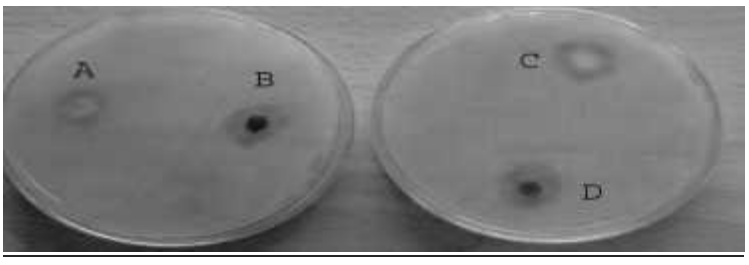
Table 4-2 : Biological activity data (zone of inhibition in mm) of Homo and co – polymers aginest fungal pathogens :

No.of polymers	codes	zone of Inhibition			
		Alternaria Solani	*Fusarium Osey Sporum*	*Mucor*	*Aspergillus niger*
Co-polymer 1	PHNBA	22	16	14	30
Co-polymer 2	PHBAC	16	13	10	25
Co-polymer 3	PANSA	21	14	16	0
Co-polymer 4	PBPA	15	15	13	15
Standard	Amphotericin-B	12	6	6	9

The result demonstrate that The co-polymers give good sinsitivity vary from (15 mm) in (PBPA) to be greater (22 mm) in co –polymer 1 (PHNBA).



(A) (B)



(C) (D)

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

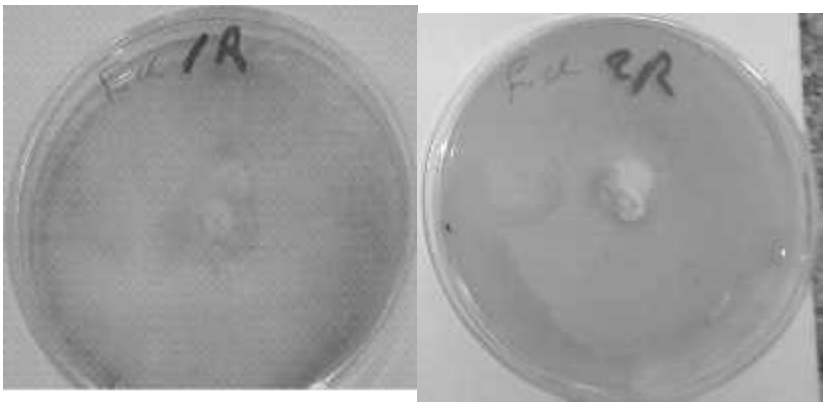
(A)Effect of (PHNBA) against *Alternaria Solani.*

(B) Effect of (PHBAC) against *Alternaria Solani* .

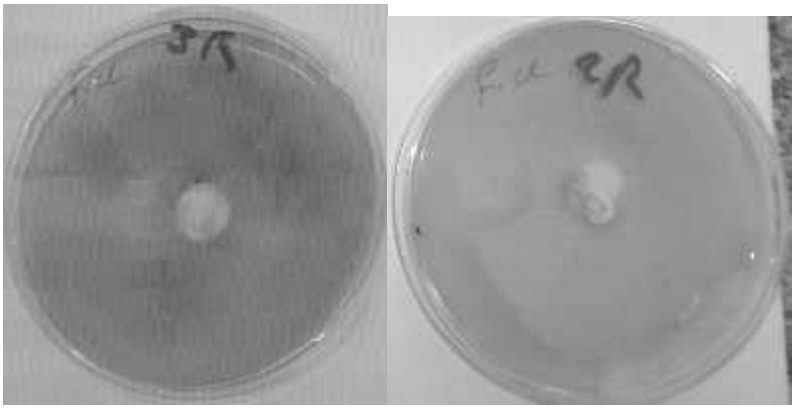
(C) Effect of (PANSA) against *Alternaria Solani* .

(D) Effect of (PBPA) against *Alternaria Solani* .

Despite that Fusarium oxseysporum shows low activity agenist (PHNBA) ,but it gives growth equal to 13 mm against (PHBAC) while the result was (14 mm) in (PANSA).



(A) (B)



(C) (D)

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

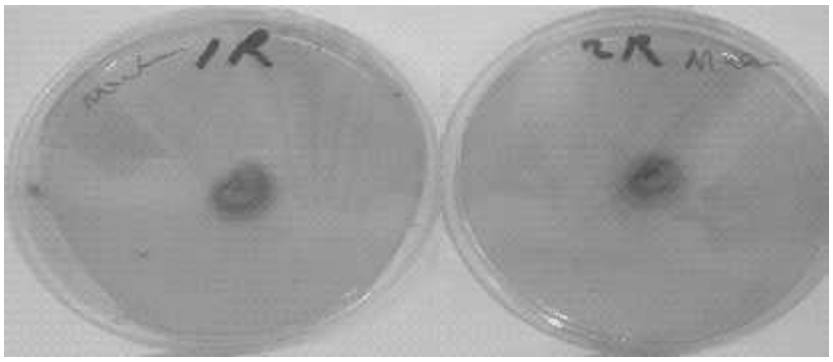
(A)Effect of (PHNBA) against *Fusarium Oxsey Sporum*.

(B) Effect of (PHBAC) against *Fusarium Oxsey Sporum*.

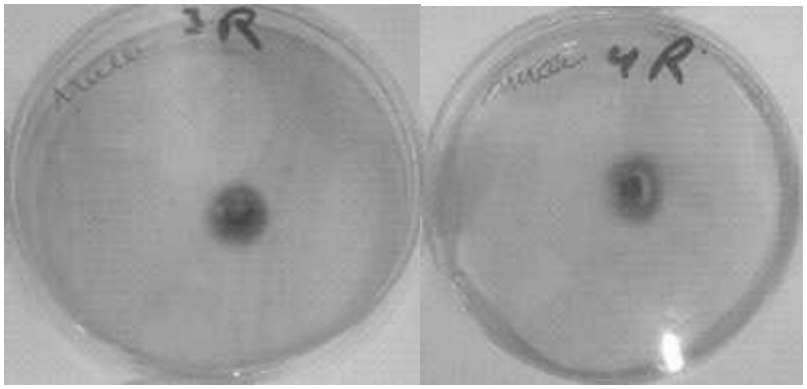
(C) Effect of (PANSA) against *Fusarium Oxsey Sporum*.

(D) Effect of (PBPA) against *Fusarium Oxsey Sporum*.

The Mucor fungi when tested to determine the antifungal activites gives the largest result aginst polymers (PANSA) reaching to 16 mm kill pathogens on solidification media ,while the less effect to be (10 mm) in (PHBAC).



(A) (B)



(C) (D)

Figure(4-7):-Statistical representation for biological activity of prepared polymers on Mucor fungi .

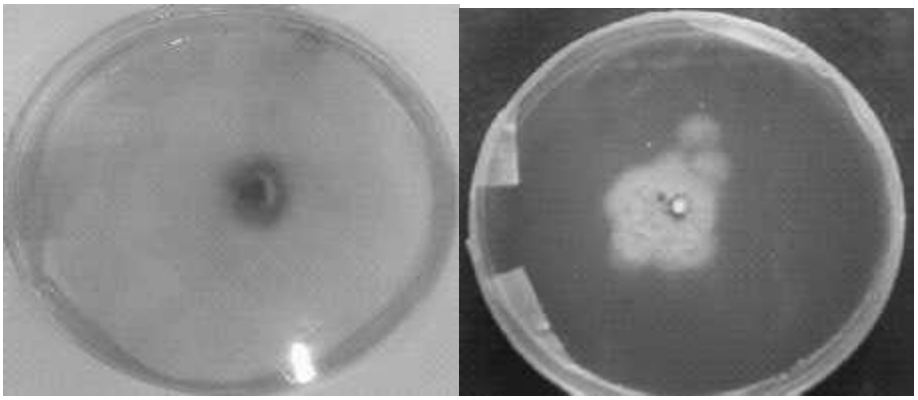
(A) Effect of (PHNBA) against Mucor fungi .

(B) Effect of (PHBAC) against Mucor fungi

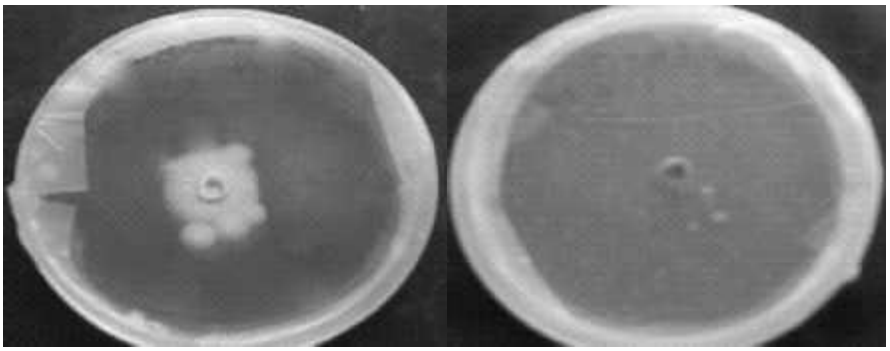
(C) Effect of (PANSA) against Mucor fungi

(D) Effect of (PBPA) against Mucor fungi.

When tested the prepared polymers on Aspergillus niger Fungi we get the higher effect by the co polymer (PANSA) that gives zero mm growth in pathogens , but in polymer (PHNBA) gives result equal to (30 mm) which can consider the lowest effect on fungi.



(A) (B)



(C) (D)

Figure(4-7):-Statistical representation for biological activity of prepared polymers on Aspergillus niger Fungi .

(A) Effect of (PHNBA) against *Aspergillus niger*

(B) Effect of (PHBAC) against *Aspergillus niger* .

(C) Effect of (PANSA) against *Aspergillus niger* .

(D) Effect of (PBPA) 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

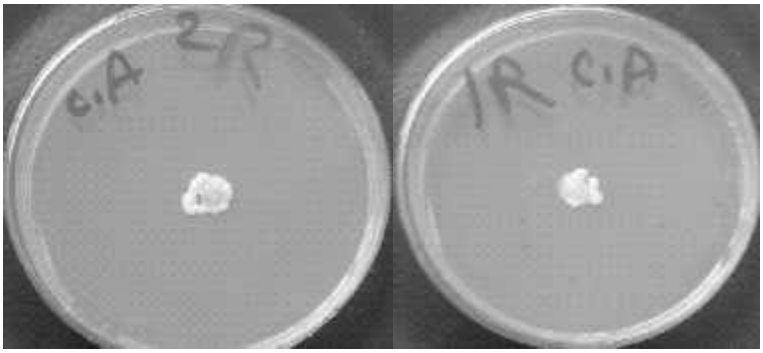
Many antifungal agents have been discovered, but only a few are active both *in vivo* and *in vitro*. Therefore, we have planned to look for new classes of newly synthesized chemical polymers. Two of the different screened newly synthesized polymers have proved to be active against tested *Candid* . 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*
Co-polymer 1	PHNBA	5	15	10	-
Co-polymer 2	PHBAC	6	17	8	-
Co-polymer 3	PANSA	3	18	12	-
Co-polymer 4	PBPA	10	11	10	1
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 (PANSA), to give the(3mm)candida albicans, while the lowest result to be (10mm) in (PBPA).



(A) (B)

(C) (D)

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

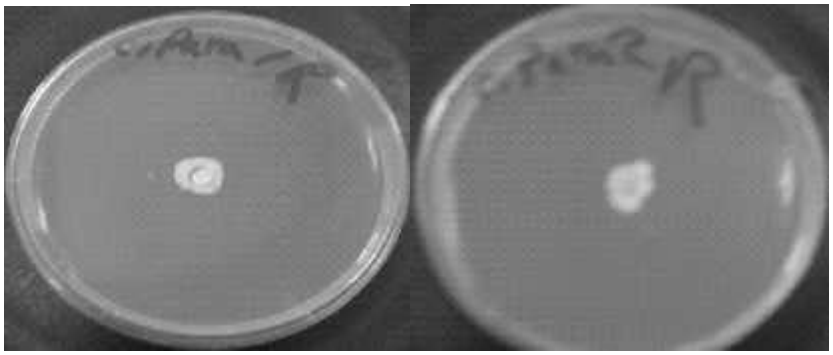
(A) Effect of (PHNBA) against *Candida albicans* .

(B) Effect of (PHBAC) against *Candida albicans* .

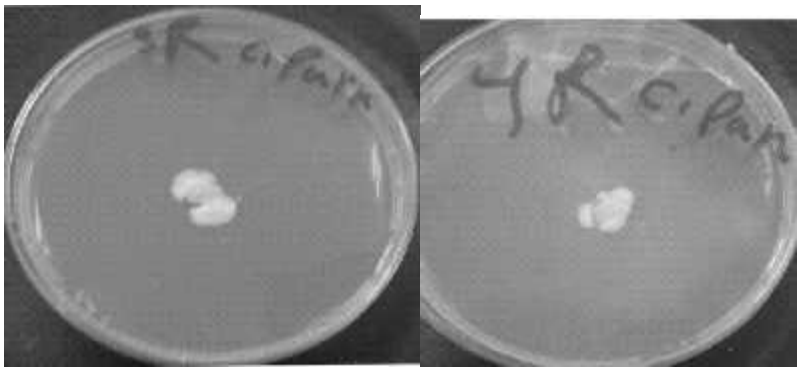
(C) Effect of (PANSA) against *Candida albicans* .

(D) Effect of (PBPA) against *Candida albicans* .

While the Candida parapsilosis shows high sensitivity to (PHBAC) that give sensitivity equal to (8mm), while the lowest result to be (12 mm) in (PANSA).



(A) **(B)**



(C) (D)

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

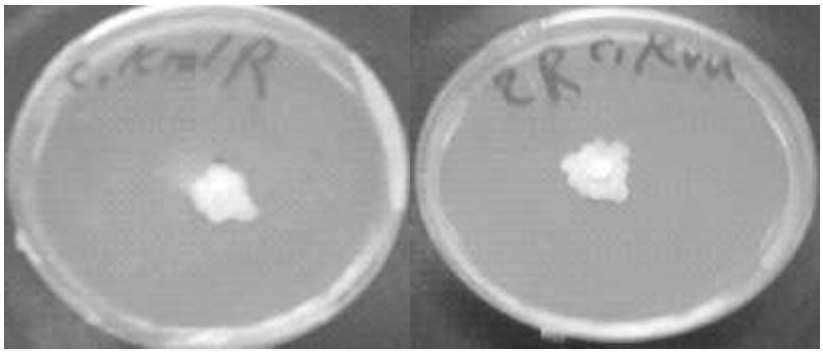
(A) Effect of (PHNBA) against *Candida Parapsilosis* .

(B) Effect of (PHBAC) against *Candida Parapsilosis*

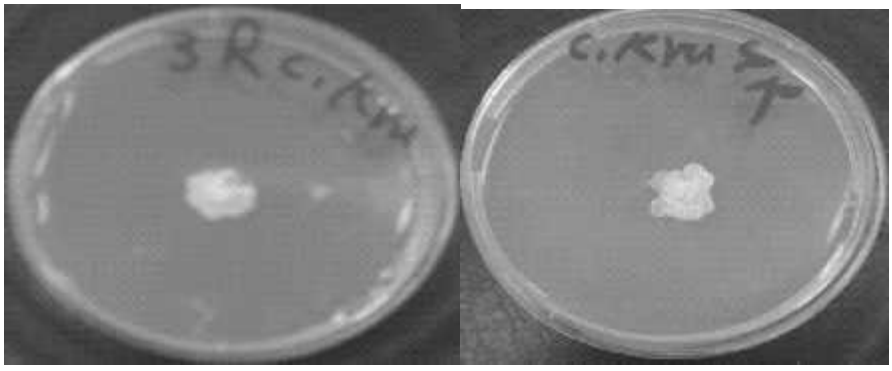
(C) Effect of (PANSA) against *Candida Parapsilosis*

(D) Effect of (PBPA) against *Candida Parapsilosis.*

where *Candida krusei showed* good effect to (PANSA) polymer, the killing percentage rises to (18 mm) ,but the remains polymers give variable results from lowest value (15 mm) in (PNAO1) polymer to reach (17 mm) in (PHBAC) polymer .



(A) **(B)**



(C) **(D)**

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

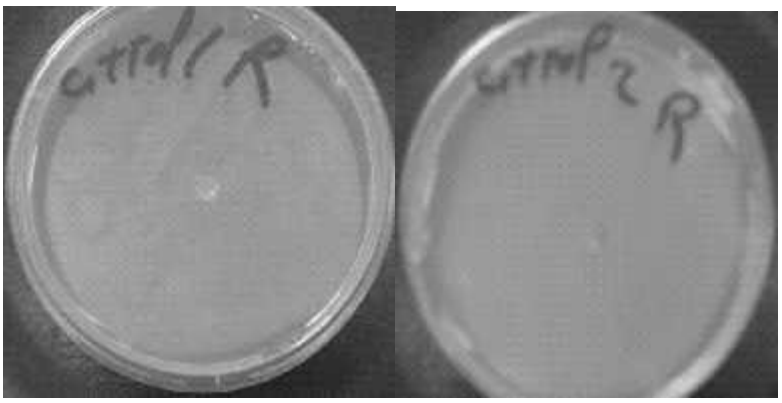
(A) Effect of (PHNBA) against *Candida Parapsilosis* .

(B) Effect of (PHBAC) against *Candida Parapsilosis*

(C) Effect of (PANSA) against *Candida Parapsilosis*

(D) Effect of (PBPA) against *Candida Parapsilosis.*

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 (PHNBA, PHBAC, PANSA) while give zero growth with killing percentage equal to 100% .



(A) (B)

(C) (D)

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

(A) Effect of (PHNBA) against *Candida Tropicalis.*

(B) Effect of (PHBAC) against *Candida Tropicalis.*

(C) Effect of (PANSA) against *Candida Tropicalis.*

(D) Effect of (PBPA) against *Candida Tropicalis.*

Finally all polymers given good sensitivity against all yeast but largest effected was shown by (PHBAC) polymers while all type of Candida growth on solidification media when treated with Candida pathogens [33].

Conclusion:

The purpose of this study was to find a new polymers to inhibit the growth of pathogenic microorganisms.The prepared polymers can inhibit the growth of the microorganisms. The activity was varied according to the tested microorganism as well as the polymer microstructure .

To explore the antimicrobial properties ,synthesized polymers was studied against :

1. ***Bacteria*** : Includes Gram-positive bacteria *Staphylococcus aureus*, and Gram-negative bacteria *Pseudomonas aeruginosa; Escherichia coli , Klebsiella pneumoniae ,* and *Salmonella typhi* , the results show the polymers high effected in both type of bacteria and the increase in concentrations of polymers lead to increase in the inhibition zone for tested antimicrobial polymers .

2. ***Fungi*** : when examined the prepared polymers against the fungi pathogens get very good activity against *Alternaria solani*, *Fusarium oxeyspurum* , *Aspergillus niger* , and *Mucor and* comparedwithStandard antifungial drug (Amphotericin-B) .

1. ***Yeast*** *:* Thesynthesized polymers are examine against yeast*such as Candida albicans* , , *candida krusei* , *candida parapsilosis* , and *candida tropicalis* show high sensitivity for most polymers than comparsion with the results obtained by the standard drug (Amphotericin-B).

From the results of biological activity possible to conclude that prepared polymers can be used in many important applications such as water purification , food Packaging, in medical fields and other applications due to have these polymers qualities of antimicoorganism Properties .

References

1. Kenawy, E.R.(2001).*J. Appl. Polym. Sci.82*, 1364.
2. Kenawy, E.R.; Abdel-Hay, F.I.; El-Shanshoury, A.E.R.; El-Newehy, M.H.(2002).*J. Polym. Sci., Part A: Polym. Chem.40*, 2384.
3. Tan, S.; Li, G.; Shen, J.; Liu, Y.; Zong, M.(2000).*J. Appl. Polym. Sci.77*, 1869.
4. Li, G.; Shen, J.(2000).*J. Appl. Polym. Sci. 78*, 676.
5. Woo, G.L.Y.; Yang, M.L.; Yin, H.Q.; Jaffer, F.; Mittelman, M.W.; Santerre, J.P.(2002).*J. Biomed. Mater. Res. 59*, 35.
6. Acharya, V.; Prabha, C.R.; Narayanamurthy, C. *Biomaterials* 2004, *25*, 4555.
7. Jiang, H.; Manolache, S.; Lee Wong, A.C.; Denes, F.S.(2004).*J. Appl. Polym. Sci. 93*, 1411.
8. Hume, E.B.H.; Baveja, J.; Muir, B.; Schubert, T.L.; Kumar, N.; Kjelleberg, S.; Griesser, H.J.; Thissen, H.; Read, R.; Poole-Warren, L.A.; Schindhelm, K.; Willcoxy, M.D.P.(2004).*Biomaterials 25*, 5023.
9. Baveja, J.K.; Li, G.; Nordon, R.E.; Hume, E.B.H.; Kumar, N.; Willcox, M.D.P.; Poole-Warren, L.A.(2004).*Biomaterials 25*, 5013.
10. Kenawy,E.R.; Worley, S.D.; Broughton R.(2007).*Biomacromolecules.8*, 1359.
11. Tiller, J.C.; Liao, C.J.; Lewis, K.; Klibanov, A.M. *Proc. Natl. Acad. Sci. U.S.A.*2001, *98*, 5981.
12. Klibanov, A.M.(2007).*J. Mater. Chem.17*, 2479.
13. Lin, J.; Qiu, S.Y.; Lewis, K.; Klibanov, A.M.(2002).*Biotechnol. Prog.18*, 1082.
14. Park, D.; Wang, J.; Klibanov, M.A.(2006).*Biotechnol. Prog. 22*, 584.
15. Majumdar, P.; Lee, E.; Gubbins, N.; Christianson, D.A.; Stafslien, .J.; Daniels,J.; VanderWal, L.; Bahr, J.; Chisholm, B.J.(2009).*J. Comb. Chem. 11*, 1115.
16. Bouloussa, O.; Rondeleza, F.; Semetey, V. (2008).*Chem. Commun.* 951.
17. Kurt, P.; Wood, L.; Ohman, D.E.; Wynne, K.J.(2007).*Langmuir*, *23*, 4719.
18. Saif, M.J.; Anwar, J.; Munawar, M.A.(2009).*Langmuir*, *25*, 377.
19. Milovic, N.M; Wang, J.; Lewis, K.; Klibanov, A.M.(2005).*Biotechnol. Bioeng.90*, 715.
20. Gottenbos, B.; van der Mei, H.C.; Klatter, F.; Nieuwenhuis, P.;

Busscher, H.J. (2002).*Biomaterials.23*, 1417.

21. Juergensen, L.; Busnarda, J.; Caux, P.Y.; Kent, R.A. (2000).*Environ. Toxicol. 15*, 174.
22. Chen, C.Z.; Beck-Tan, N.C.; Dhurjati, P.; Van Dyk, T.K.; LaRossa, R.A; Cooper, S.L.(2000).*Biomacromoles.1*, 473.
23. Kocer, H.B.; Worley, S.D.; Broughton, R.M.; Huang, T.S.*(* 2011*). React. Funct. Polym.*, *71*, 561.
24. Worley, S.D.; R. Wu, F. Li; Kim, J.; Wei, C.I.; Williams, J.F.; Owens, J.R.; Wander, J.D.; Bargmeyer, A.M.; Shirtliff, M.E.(2003).*Surf. Coat. Int. Part B, Coat. Trans.*, *86*, 273.
25. Sun, Y.; Sun, G.(2001).*J. Polym. Sci., Part A: Polym. Chem. 39*, 3348.
26. Baveja, J.K.; Li, G.; Nordon, R.E.; Hume, E.B.H.; Kumar, N.; Willcox, M.D.P.; Poole-Warren, L.A.(2004).*Biomaterials 25*, 5013.
27. Hume, E.B.H.; Baveja, J.; Muir, B.; Schubert, T.L.; Kumar, N.; Kjelleberg, S.; Griesser, H.J.; Thissen, H.; Read, R.; Poole-Warren, L.A.; Schindhelm, K.; Willcoxy, M.D.P.(2004).*Biomaterials 25*, 5023.
28. Acharya, V.; Prabha, C.R.; Narayanamurthy, C.(2004).*Biomaterials*, *25*, 4555.
29. Woo, G.L.Y.; Yang, M.L.; Yin, H.Q.; Jaffer, F.; Mittelman, M.W.; Santerre, J.P. *J.(* 2002*). Biomed. Mater. Res. 59*, 35.
30. Li, G.; Shen, J.(2000).*J. Appl. Polym. Sci*., *78*, 676.
31. Dikic,T.; Erich, S.J.F.; Ming, W.; Thüne, P.C.; Huinink, H.P.; van Benthem; R.A.T.M.; de With, G.(2007).*Polymer, 48*, 4063.
32. Dikic,T.; Ming, W.; Thüne, P.C.; van Benthem, R.A.T.M.; de With, G.(2008).*J. Polym. Sci., Part A: Polym. Chem.46*, 218.
33. Narendra P. S. Chauhan,1 Rakshit Ameta,2 Suresh C. Ameta ,(2008). Journal of Applied Polymer Science,MohanLalSukhadiaUniversity,. Udaipur 313 001, Rajasthan, India.
34. A.-G. Mahmoud Yehia, M. Aly Magda ,(2004). Anti-Candida and mode of action of two newly synthesized polymers, *Mycopathologia* 157: 145–153.
35. Narendra P. S. Chauhan, Rakshit Ameta ,*(2010). Malaysian Polymer Journal, Vol. 5, No. 2, p 162-180 .*
36. A.-G. Mahmoud Yehia, M. Aly Magda ,(2004). Anti-Candida and mode of action of two newly

synthesized polymers, *Mycopathologia* 157: 145–153.

37. Witte, W.(2008).*Science*, 279, (5353), 996-997.
38. Chang, Y.; McLandsborough, L.; McClements, D.J.(2012). Cationic antimicrobial (ε-polylysine)-anionicpolysaccharide (pectin) interactions: Influence of polymer charge on physical stability and antimicrobial efficacy. *J. Agric. Food Chem. 60*, 1837–1844.
39. Zhang, C.; Zhu, Y.; Zhou, C.; Yuan, W.; Du, J.(2013). Antibacterial vesicles by direct dissolution of a block copolymer in water. *Polym. Chem.4*, 255–259.

تخليق وتشخيص انواع جديدة من البوليمرات المضادة للجراثيم ودراسة فعاليتها البايولوجية

تاريخ الاستلام : 2015/1/7 تاريخ القبول :2015/3/3

رافد قيس كمال

قسم الكيمياء - كلية التربية - جامعة القادسية

الخلاصة:

يصف هذا البحث تخليق انواع جديدة من البوليمرات المشاركة التي يمكن تخليقها من تفاعل (تلوين حامض السلفونيك , 2,4-داي كلورو حامض البنزويك, بارا-هايدروكسي حامض البنزويك, الانلين وبارا برومو فينول) مع (هايدروكينون, كلورو حامض البنزويك , 5-سلفو حامض السالسليك, وبارا برومو انلين) على التوالي والفورمالديهايد بوجود حامض الهايدوكلوريك كعامل مساعد, وقد تم تشخيص هذه البوليمرات المضادة للجراثيم بواسطة التحليل الطيفي FT-IR , حيث اظهرت جميع البوليمرات التي تم تخليقها فعالية نشطة مضادة للجراثيم البكتريا, الفطريات والخمائجميع البوليمرات المخلقة اظهرت فعالية جيدة مضادة للجراثيم مقارنة مع السيبروفلوكساسين و بـ الامفوتريسين كعقاقير قياسية.

الكلماتالمفتاحية: بوليمرات مضادة للجراثيم , الفعالية البايولوجية , بوليمرات مشاركة جديدة