

## Preparation and Evaluation of PolyVinyl Alcohol Derivative as Antimicrobial and Antifungal Agents.

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### Abstract:

Polyvinyl alcohol was Cynoethylated , complex compound with Iodin in presence of  $Cu^{++}$  ions were prepared and their ultra violet (U.V) and infra red( IR) spectra were investigated.

The prepared derivative and complexes were evaluated as antibacterial and antifungal agents following the standard dilution method. MIC(minimum inhibitory concentration) for each polymer using ten types of gram + ve and gram - ve bacteria were determined in addition to three types of fungi. The results obtained showed that MIC's were around  $0.0011 \times 10^3$  molar for different polymetric derivatives tried.

### Introduction:

Now days polymeric substances have attained invaluable important in medicine as well as in nearly every sphere of life. Their relatively easy variability of physical and chemical properties enables accommodation to many different requirement.

Only during the last two decades it has been recognized that the interaction of synthesis polymers with biological materials can also be favorable and desirable .Thus, polyglycolide and polylactide , which are applied as sutures materials in surgery, are slowly degraded and resorbed in the body.

Definite pharmacological properties have been discovered in the case of other polymers. Polymers can thus, provide a safe ( not inherently toxic) universal platform for administering biological compounds which need amore controlled delivery (1).

Ikada *et al.* (1984) synthesized polymers of quaternary ammonium salt surfactants and observed their antibacterial activity (2).

Senuma *et al.*(1989)have investigated copolymerization of acrylonitrile and substituted styrenes which are derived from chloromethyle styrenes tertiary amines and have examined the antibacterial activity of the copolymers.

It was also found that where antibacterial agent like penicillin is linked to poly(Vinyl amine), its about 30-40 times longer lasting than that of free pencillin (4).

In this connection , we have prepared some derivatives of the water soluble poly (Vinyl alcohol) and examined the antibacterial and antifungal activities of the prepared entities.

Poly vinyl alcohol (PVA) ,a polyhydroxy polymer, is the largest volume , synthetic , water –soluble resin produced in the world .It is commercially produced by the hydrolysis of poly vinyl caetate , as the vinyl alcohol monomer does not exist in the free state, although traces have been detected (5).It is with

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molecular weight about 14.000 , So the hydrolysis and molecular weight have effect on the properties of Poly vinyl alcohol leads to variations in this properties depends on the increasing or decreasing of molecular weight at a constant degree of hydrolysis and increasing of or decreasing of hydrolysis at a constant molecular weight(6).

## **Material And Methods:**

### **1-Materials :**

All chemical used were obtained from BDH Chemical Ltd U.K.,and they are analytical grade.

Biomaterials were obtained from oxide or Biomerieux Ltd.

### **2-Methods:**

#### **2.1 Cynoethylation of poly vinyl alcohol.**

A slurry of 0.00032 mole polyvinyl alcohol (PVA) and 0.5 mole acrylonitrile (AN) and 0.5% aqueous of sodium hydroxide was refluxed under continuous stirring for 45 min.the reaction mixture was then cooled and neutralized with glacial acetic acid.

The product was poured in to about 0.2 L diethyl ether a taffy like precipitate was obtained, the precipitate was further purified by dissolving in 300 ml of acetone and again precipitated by pouring into diethyl ether .The product was then dried in vacuum oven until constant weight in order to let the yield was almost quantitative.

The polymer was characterized by elemental analysis , infrared and ultraviolet spectra.

#### **2.2 Preparation of -A-Iodine complex:**

The method used was a modified one based on method of Toshihiro *et al.*(1986) : 0.5 gm PVA was dissolved in 5 ml distilled water .0.4 gm sublimed Iodine

one 0.16 gm NaOH was added and the mixture was heated at 25-30 C° under continuous stirring for two hours., the reaction mixture was cooled kept in refrigerator over night and kept in brown bottle and used for antimicrobial and antifungal test the material was characterized by ultraviolet and infrared spectra.

#### **2.3 Preparation of complex compound between PVC,Copper II and I<sub>2</sub>.**

The method followed was to add copper (II)nitrate and Sodium hydroxide to a solution of 0.5 PVA in 5 ml distilled water.

Deep blue color solution was obtained .which was changed into a green color. Then sublimed I<sub>2</sub> was added and the method was continued as described above.

The product was characterized by infrared and ultraviolet spectra.

#### **2.4 Evolution of Antimicrobial activity:**

##### **Antimicrobial Susceptibility Testing:**

Antimicrobial susceptibility tests measure the ability of an antimicrobial agent to inhibit or to kill bacterial growth *in vitro*. This ability may be estimated by either the dilution method or the diffusion method.In this work the broth dilution method was followed.

Graded amount of antimicrobial substances are incorporated into liquid or solid bacterial media.The media are subsequently inoculated with test bacteria and incubated. The end point is taken as that amount of antimicrobial substance required to inhibit the growth of - or to kill- the test bacteria.

**Procedure:****A-Isolation :**

Ceratin bacterial isolates were chosen , having in mind that they represent both (G +Ve) gram positive and( G-Ve) gram negative bacteria. The selected organisms were *Escherichia coli* , *Salmonella typhii* and *Paratyphoid* , *Shigella dysentery* and *Pseudomonas aeruginosa* representing G- Ve while *Staphylococcus aureus*, *clostridium welchii* and *Bacillus subtilis* were representing the G +Ve. Those isolates were taken from patients at ( Central Public Health Laboratory) .

**B-Stock Chemical Solutions :**

Dissolved 0.75 gm from the tested polymer in 10 ml dilute solution ( containing 8 ml distilled water and 2 ml ethanol).

**Culture:**

1-Separate colonies from purified and identified isolated bacteria hours culture and incubated at 37 C° were selected and then cultured on both media ( brain heart infusion, or tryptic soy).

2-One ml of culture prepared of the selected isolates was added to 9 ml of new fresh broth or normal saline.

3-Dilution of the polymer were done to used as antimicrobial and antifungal reagent at the concentrations of 0.066 gm/ml , 0.049 / ml, 0.033 gm/ ml , 0.01 gm/ml , 0.013gm /ml , 0.005 gm/ ml, 0.005 gm /ml of the polymer.

4-To each of the above prepared polymer tubes, 1 ml broth of the prepared required organism was added .

5-The tubes incubated at 37 C° for 18 hours.

6-After 18 hour , sample was taken by a loop from the tubes and distributed uniformly on the agar media prepared in a petri-dishes (90 mm diameter ). This was repeated for all concentration of the agent.

7-The dishes were kept for 18 hours incubation , then investigated visually to determine the inhibition of growth completely or partially.

The MIC is defined as the lowest concentration of antimicrobial agent at which there is no visible growth .Any hazy growth was ignored.

Moreover , one or two colonies on the spot with the agar method was also ignored.

N.B.A.blank experiment was carried out in the same manner using the dilute alone (ethanol ; distilled water). Without adding the antimicrobial agent.

**2.5 Evolution Of Antifungal Activity:**

In this work the culture of the organism method was followed. Dilution susceptibility test followed for antibacterial evaluation was applied also for testing the activity of antifungal agents against .(*Candida albicans* ; yeast like candida and *Alternaria*).

MIC values were determines in the same manner for each agent examined however, the concentrations used with antifungicides were as follows .( 0.066 gm/ ml , 0.049 gm/ml , 0.033 gm/ ml , 0.0166 gm/ ml , 0.013 gm/ ml, 0.005 gm/ml , 0.005 gm / ml).

**Results and Discussion:**

In tables (1, 2 ,3) are tabulated results obtained on using three prepared polymer derivatives as antimicrobial agents. According to the MIC values determined the polymers screened can be arranged in the following order.

Cu / I<sub>2</sub>/PVC complex > I<sub>2</sub>PVC > Cyno Ethylated PVC.

The mechanism of action of most antimicrobial druges is not completely understood. However , the mechanism of action can be placed under five heading (8 , 9 , 10 ).

1- Inhibition of cell wall syntheses.

2- Inhibition of cell wall membrane function.

3- Inhibition of protein syntheses (i.e.) inhibition of translation and transcription of genetic material.

4- Inhibition of nucleic acid syntheses.

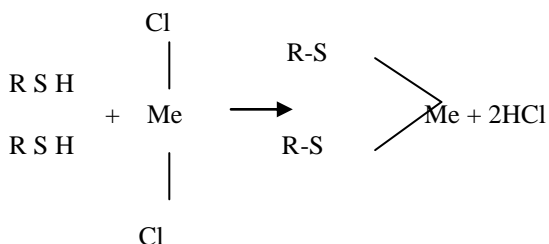
5- Competitive antagonism of same metabolite.

6- On complexing PVA with I<sub>2</sub> and metals the activity of the polymer against bacteria was profoundly increase and MIC was change from 0.066 gm/ml , 0.049 gm/ ml , 0.033 gm /ml, 0.0166 gm / ml , 0.013 gm / ml , 0.006 gm/ ml , 0.005 gm / ml.

The possible made of action of metal containing compounds may be through removal of sulfhydryl groups (11). Enzyme protein containing cysteine have side chains termination in sulfhydryl groups.

Delivery of complexed iodine to the sensitive elements of the cell membrane to be the crucial events of antibacterial action (11 , 12). Iodine targets are located in the bacterial cytoplasm and cytoplasmic membrane and its killing action takes place in a matter of seconds. In contact with polymer\_bound iodine , sulfhydryl compounds , peptides , proteins , enzymes , vitamine C , lipids and cytosine are iodinated and oxidaed by free iodine resulting in inactivation of molecules that are essential for biological viability (11,12).

Many metals like wise interfere combining with sulfhydryl groups (13).



For this copper and iron are extremely toxic.

### Antifungal activity of the polymeric agent :

The results of investigation of testing the polymer drugs prepared against three types of fungi collected in table (4),(5) and (6).

The candida , yeast and alternia was found to be completely resistant to cyanoethylated PVA, irrespective of the fact that it was successful as antibacterial agent. However ,poly vinyl lcohol-iodine and polyvinyl alcohol-copper – iodine are shown to be microbacterial agents and act on a wide variety of bacteria (table 2-3).Polyvinyl alcohol , the hydrophilic polymer that acts as a carrier for iodine dose not have any intrinsic antimicrobial activity , but by virtue of its affinity to cell membranes, it delivers diatomic free-iodine (I<sub>2</sub>)directly to the microbial cell surface .Similar results were found for polyvinylpyrrolidone iodine (14).

### Infrared Spectra:

The spectrum of polyvinyl alcohol (fig.1)shows clear evidence of the presence of hydroxyl groups from the relatively intense band near 3500 cm<sup>-1</sup> as polyvinyl alcohol is a secondary alcohol , the C-O frequency of the C-OH group occurs at 1400cm<sup>-1</sup> is also obtained Cyanoethylation lead to the introduction of nitrile group and appearance of nitrile absorption band at about 2200cm<sup>-1</sup> the region in which polyvinyl alcohol is relatively transparent (15).(fig 3) .In the spectra of Cu and Cu/ I<sub>2</sub> complexes and adducts,(figs. 3-4) the absorption at 800-1020cm<sup>-1</sup> , characteristic of complex and adduct structure , occurred (16).

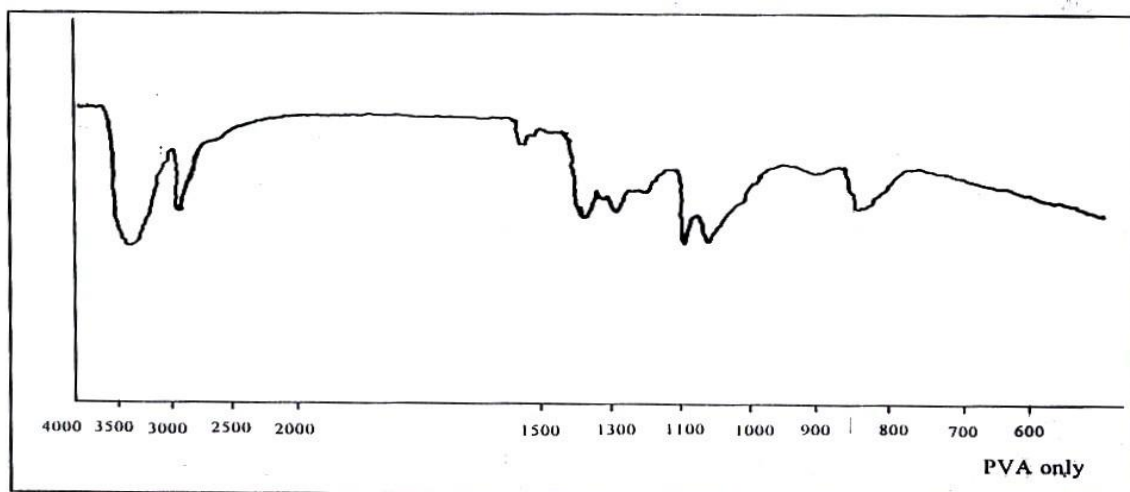


Figure (1):Infrared spectrum of Polyvinyl alcohol.

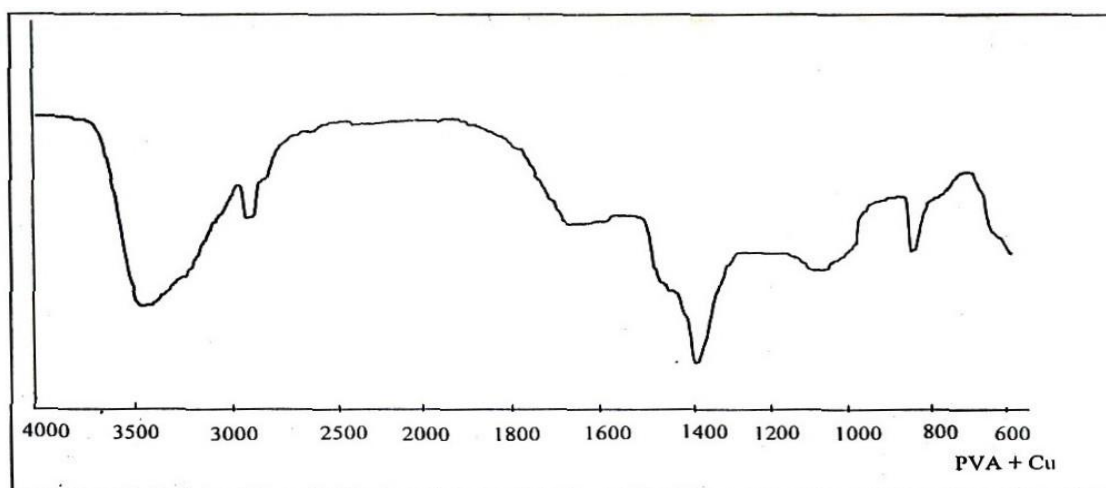


Figure (2):Infrared spectrum of Polyvinylalcohol +Cu.

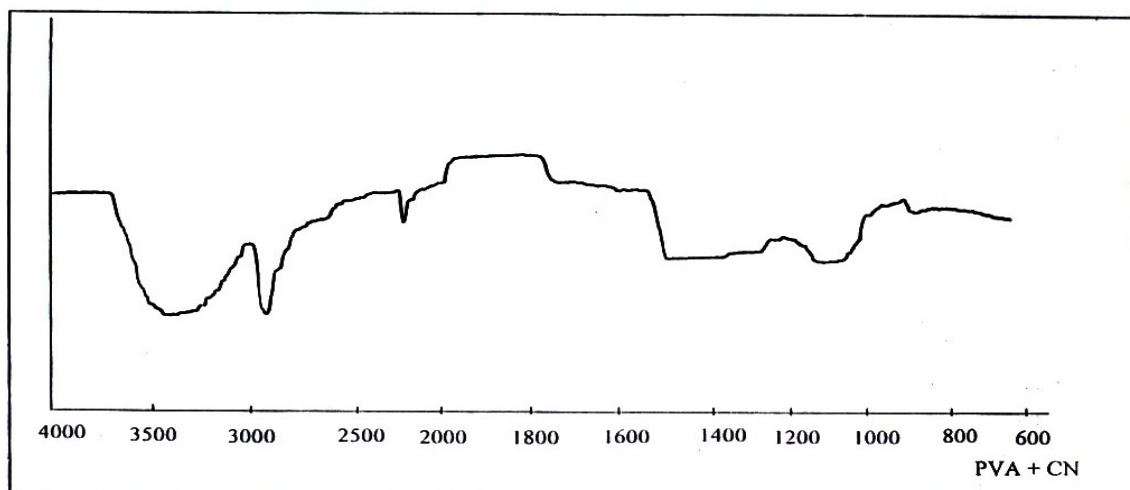


Figure (3):Infrared spectrum of Polyvinyl alcohol +CN.

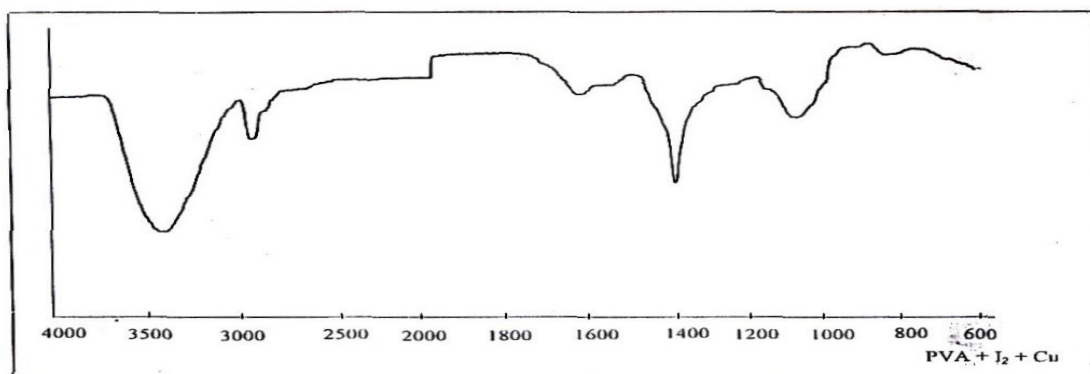


Figure (4):Infrared spectrum of Polyvinylalcohol +I<sub>2</sub>+Cu.

**Ultraviolet Spectra:**

Fig.(5) show ultraviolet spectrum of poly (vinyl alcohol alone).It shows maximum absorption at  $\lambda = 202.3$  nm and  $\lambda = 279.4$  nm.

On formation of complex with Cu , salt , absorption is moved to the visible region at 612.1 nm.fig.(6) show the U:V-Visible spectra for PVA /Cu/ Iodine

compound :the absorption peak is now at 578.2 nm.

This absorption band was show to be due to iodine complex or bound iodine and as can be seen from Fig.(7) it can present in the U.V.spectrum of only PVA plus iodine in alkaline media.

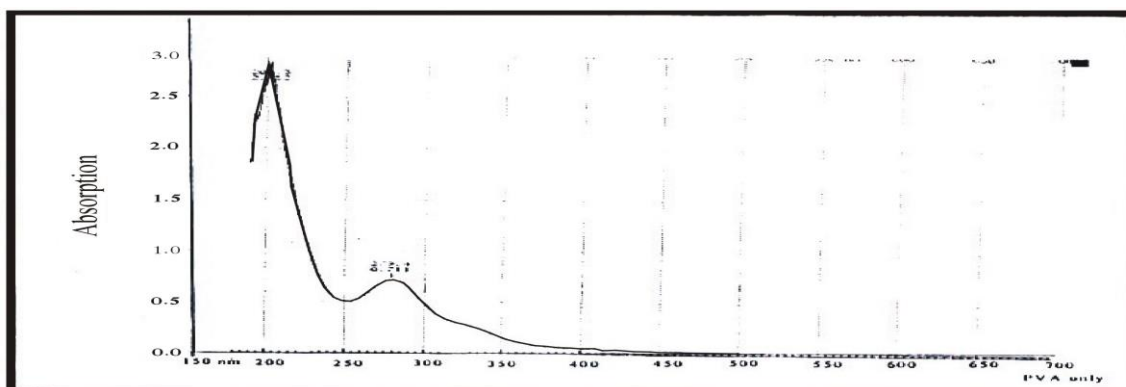


Figure (5):Ultraviolt spectrum of Poly vinyl alcohol .

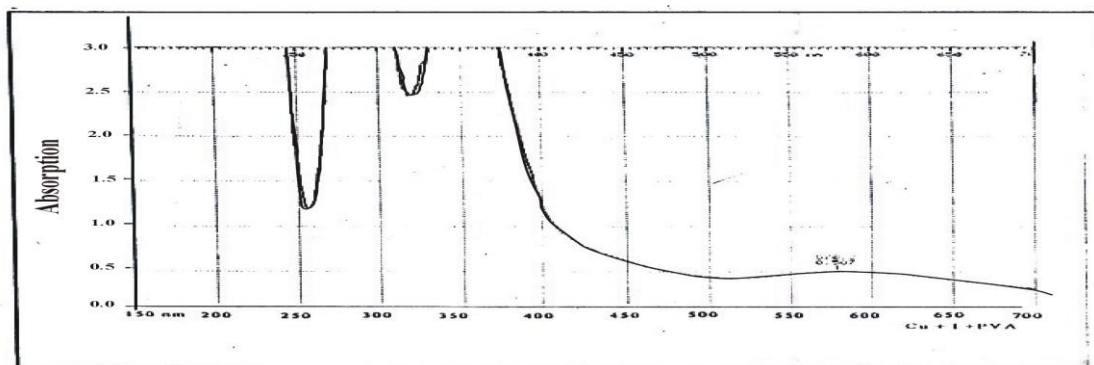


Figure (6):Ultraviolt spectrum of Polyvinyl alcohol +I<sub>2</sub>+Cu .

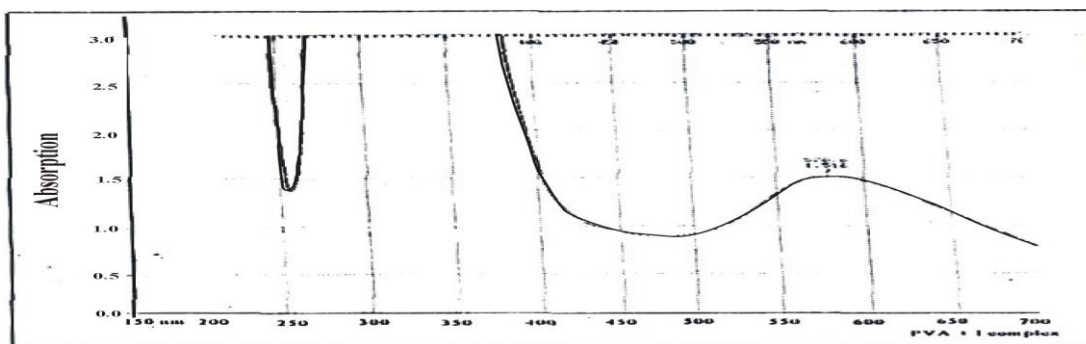


Figure (7):Ultraviolet spectrum of Poly vinyl alcohol +I<sub>2</sub>.

Table(1): Effect of Gyno Ethylated Poly Vinyl alcohol (CEPVA)on the Growth of Selected Bacterial Isolates.

Isolates	Gram strain	Concentration of Antibacteria gm/ml.				
		0.066	0.049	0.033	0.0166	0.013
<i>Escherichia Coli</i>	G-Ve	-	-	-	-	+
<i>Salmonella typhi Paratyphi</i>	G-Ve	-	-	-	-	+
<i>Shigella dysentery</i>	G-Ve	-	-	-	-	+
<i>Pseudomonas aeruginosa</i>	G-Ve	-	-	-	-	+
<i>Klebsiella pneumoniae</i>	G-Ve	-	-	-	-	+
<i>Neisseria gonorrhoea</i>	G-Ve	-	-	-	-	+
<i>Staphylococcus aureus</i>	G+Ve	-	-	-	-	+
<i>Bacillus subtilis</i>	G+Ve	-	-	-	+	+

(-) no growth

(+) growth

Table(2): Effect of PolyVinyl alcohol / Iodine complex (PVA/ I) on the Growth of Selected Bacterial Isolates.

Isolates	Gram strain	Concentration of Antibacteria gm/ml.						
		0.066	0.044	0.033	0.0166	0.013	0.008	0.004
<i>Escherichia Coli</i>	G-Ve	-	-	-	-	-	-	+
<i>Salmonella typhi Paratyphi</i>	G-Ve	-	-	-	-	-	-	+
<i>Shigella dysentery</i>	G-Ve	-	-	-	-	-	-	+
<i>Pseudomonas aeruginosa</i>	G-Ve	-	-	-	-	-	-	+
<i>Klebsiella pneumoniae</i>	G-Ve	-	-	-	-	-	-	+
<i>Neisseria gonorrhoea</i>	G-Ve	-	-	-	-	-	-	+
<i>Staphylococcus aureus</i>	G+Ve	-	-	-	-	-	-	+
<i>Bacillus subtilis</i>	G+Ve	-	-	-	-	-	-	+

(-) no growth

(+) growth

Table(3): Effect of PolyVinyl alcohol / Copper-Iodine complex (PVA/Cu- I) on the Growth of Selected Bacterial Isolates.

Isolates	Gram strain	Concentration of Antibacteria gm/ml.							
		0.066	0.049	0.033	0.0166	0.013	0.008	0.004	0.002
<i>Escherichia Coli</i>	G-Ve	-	-	-	-	-	-	-	+
<i>Salmonella typhi Paratyphi</i>	G-Ve	-	-	-	-	-	-	-	+
<i>Shigella dysentery</i>	G-Ve	-	-	-	-	-	-	-	+
<i>Pseudomonas aeruginosa</i>	G-Ve	-	-	-	-	-	-	-	+
<i>Klebsiella pneumoniae</i>	G-Ve	-	-	-	-	-	-	-	+
<i>Neisseria gonorrhoea</i>	G-Ve	-	-	-	-	-	-	-	+
<i>Staphylococcus aureus</i>	G+Ve	-	-	-	-	-	-	-	+
<i>Bacillus subtilis</i>	G+Ve	-	-	-	-	-	-	-	+

(-) no growth

(+) growth

**Table(4): Effect of Cynothylated PVA (CEPVA) on the Growth of Selected fungal Isolates.**

Isolates	Concentration of Antifungal gm/ml.				
	0.066	0.049	0.033	0.0166	0.013
<i>Candida albicans</i>	+	+	+	+	+
<i>Yeast like fungal</i>	+	+	+	+	+
<i>Alternaria</i>	+	+	+	+	+

(+)growth

**Table(5): Effect of polyvinal alcohol /Iodine complex (PVA/I) on the Growth of Selected fungal Isolates.**

Isolates	Concentration of Antifungal gm/ml.					
	0.066	0.049	0.033	0.0166	0.013	0.008
<i>Candida albicans</i>	-	-	-	-	-	+
<i>Yeast like fungal</i>	-	-	-	-	-	+
<i>Alternaria</i>	-	-	-	-	-	+

(-)no growth

(+)growth

**Table(6): Effect of polyvinal alcohol / Copper –Iodine complex (PVA/Cu-I) on the Growth of Selected fungal Isolates.**

Isolate	Concentration of Antifungal gm/ml						
	0.066	0.049	0.033	0.0166	0.013	0.008	0.005
<i>Candidacians albicans</i>	-	-	-	-	-	-	+
<i>Yeast like fungal</i>	-	-	-	-	-	-	+
<i>Alternaria</i>	-	-	-	-	-	-	+

(-) no growth

(+) growth

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## تحضير و تقييم مشتقات البولي فنيل الكحول كمضادات للبكتريا و الفطريات

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### الخلاصة:

تم تحضير مشتقات البولي فنيل الكحول السيانواثيلينية كذلك معقداته مع النحاس الثنائي ، ومعقداته مع النحاس الثنائي و البيود .شخصت هذه المشتقات بواسطة الأشعة تحت الحمراء و فوق البنفسجية . قيمت المشتقات و المعقدات المحضرة كمضادات للبكتريا و الفطريات باتباع طريقة التخفيف القياسية و عينت قيمة MIC (اقل تركيز مثبت ) لكل مشتق و باستخدام عشرة أنواع من البكتريا وثلاثة أنواع من الفطريات . و بينت النتائج أن قيمة MIC تتمحور حول  $0.0011 \times 10^3$  مولار لأنواع البوليميرات المختلفة المدروسة .