SYNTHESIS, CHARACTERIZATION AND STUDY THE EFFECT OF (3,5-DIMETHYL-1H-PYRAZOL-4- YL) MERCURY (II) CHLORIDE ON GROWTH INHIBITION OF SOME BACTERIA ,YEAST AND SOME FUNGI (*IN VITRO*). .

Eman.T.Ali

Department of drugs and toxicology, college of pharmacy, university of Basrah,Basrah (Iraq).

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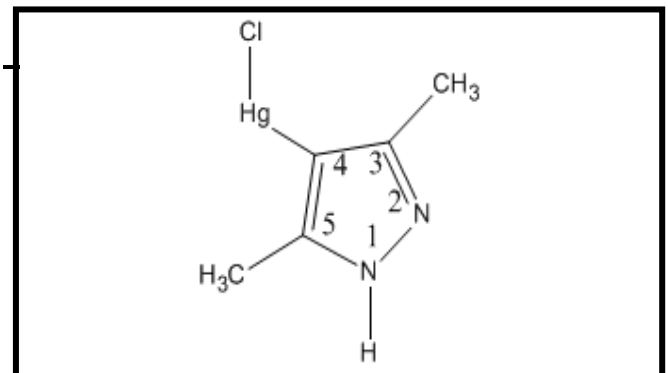
Key words:- Gram-positive bacteria, gram-negative bacteria, yeast.

ABSTRACT

The reaction of 3,5-dimethyl-1H-pyrazole with mercury (II)acetate in absolute methanol gave (3,5-dimethyl-1H-pyrazol-4-yl) mercury(II) chloride (DMPMC), which characterized by microanalysis, ^{1}H, and ^{13}C-NMR and IR. It was found that 0.22M of (DMPMC) dissolved in ethanol/water added to Muller-Hinton Agar medium and Sabourauds-Dextrose agar medium (SDA) respectively, inhibited the growth of some gram negative bacteria **[***Escherichia-coli, Klebsiella-aerogenes and Pseudomonas-aeruginosa*], also gram positive bacteria [*Staphylococcus-aureus, Streptococcus-pyogenes*] and Candida-albicans as well as some fungi [*Aspergillus-flavus, Aspergillus-fumigatus and Aspergillus-niger*]. Higher concentrations of (DMPMC) solution in to the media inhibited growth of bacteria yeast and fungi under studies more strongly. The minimal inhibitory concentration (MIC)and the cytotoxicity of (DMPMC) were studied against human being blood , it was found that it has no haemolysis at different concentrations in vitro.

INTRODUCTION

It is well known that pyrazole derivatives ,which is five-membered heterocyclic compound having two adjacent nitrogenes and two double bonds[(1)] . Many derivatives of these systems were used as drugs[(2)], Phenyl butazone used for relieving pain in acute gout and in the treatment of spondylities and in rheumatoid arthritis, Oxyphenbutazone it has some therapeutic uses as that of phenylbutazone, but it causes less gastric irritation. Phenazone (Antipyrine) it is potent analgesic and antipyretic, it also possesses anti-inflammatory activity. It is used in influenza fever. Dithiocarbazate complexes which contain two adjacent nitrogen atoms and two sulfur atoms had some biological activity, it was used as anticancer[(3)] ,antibacterial, antifungal[(4)] and antiviral[(5)]. Selenadiazoles possesses some physiological and medical effect[(6,7,8,9)]. The bacterial, yeast and fungal action of diazoles (pyrazoles) containing mercury have not been studied. The aim of the present work is to prepare a mercurial derivatives for 3,5-dimethyl-1H-pyrazole and to study the bacterial action of (DMPMC) on some gram positive and gram negative bacteria , yeast as well as some fungi, and study the (MIC) and the cytotoxicity of the new compound as well as the ability to use it as antiseptic. This topic, to our knowledge has not been investigated.



(3,5-dimethyl-1*H*-pyrazol-4-yl)mercury(II) chloride

Synthesis of (3,5-dimethyl-1H-pyrazol-4-yl)mercury (II) chloride:-

(14.55g, 0.15mol) of 3,5-dimethyl-1H-pyrazole and (47.64g, 0.15 mol) of mercury(II)acetate were refluxed in 500ml round bottomed flask for seven hr and thirty minutes using 200ml of absolute methanol as a solvent, cool to room temperature 25Cº then (7.21g, 0.17mol) of LiCl which dissolved in 50ml absolute methanol was added to the mixture with stirring for another one hr, filter off and collect the white precipitate on the Buchner funnel. Wt. 44.50g , yield =89% , m.p = 178 C with decomposition.

Elemental analysis:-

Calculated : C : 18,13 , H : 2.13 , N : 8.46

Found : C :(18.11) , H :(2,12) , N :(8.42)

Materials and Methods:-

3,5-Dimethyl-1H-pyrazole was obtained from Fluka and mercurated with mercuric acetate in absolute methanol was used in this study. Standard solution of (3,5-dimethyl-1H-pyrazol-4-yl)mercury(II)chloride (DMPMC) was prepared by dissolving (0.732g) of (DMPMC) in 10ml of ethanol/water (0.22M), different concentrations (0.1, 0.05, 0.04, 0.03, 0.02M) of (DMPMC) were used.

Experimental -A-

All bacterial strain (*Staphylococcus aureus* and *Streptococcus pyogenes* isolates from Vaginal swab, *Escherichia coli* isolate from reference ATCC25922, *Pseudomonos aeruginosa* and *Klebsiella aerogenes* from blood culture). Yeast (*Candida albicans*) from Vaginal swab. were collected, identified. The (DMPMC) activity were tested on pathogens isolates by disc diffusion method [10] using Muller-Hinton agar media (Oxoid) of a depth (4mm) in standard Petri dish (90mm diameter) inoculated with 10^5 CFU/ml bacterial suspension by dispersion method (according to McFarland standard scale)[11] , then the Petri dishes were left after inoculation for (10-15) minutes. Six units of sterilized paper disc (5mm diameter) saturated with the above concentrations of (DMPMC) were put in the bottom of the Petri dishes, Lightly touch each disc with sterile inoculating forceps to make sure that it was in good contact with agar surface, incubated upside down at 37Cº for 24 hours to measure the inhibition zone. Six Petri dishes were used as an experimental unite and the trial was repeatedtwice. .

Experimental-B-

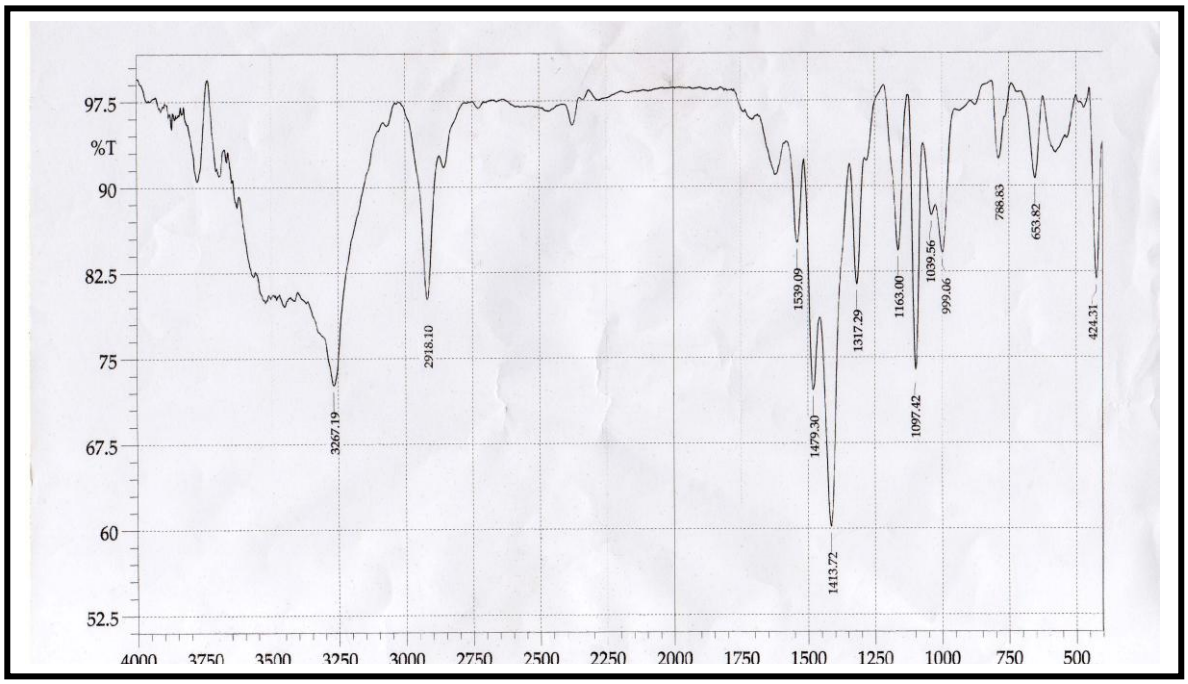
Soil samples were taken from the depth of (15-25) Cm, placed in plastic bags and subsequently brought to the laboratory isolation of fungi was made using Potato Carrot Agar (PCA) in Petri dishes by sprinkling (1g) of each soil sample on the surface of agar medium and incubating at 25Cº. Petri dishes were examined after (5-7) days for any fungal growth [12]. All Fungi (*A. flavus*, *A. fumigatus* and *A.niger*) were isolated and identified [13,14]. The (DMPMC) activity were tested on Fungi by disc diffusion method[15] using Sabourauds-Dextrose Agar medium (SDA), Six units of sterilized paper disc (6mm diameter) saturated with above concentrations of (DMPMC) were put in the bottom of the Petri dishes which contain 20ml of (SDA) which solidified and 0.2ml of suspension Fungi 10^5CFU/ml were dispersion on each Petri dish, incubated at 25Cº for (1-2) days to measure the inhibition zone. Four Petri dishes were used as an experimental units and the trial was repeated twice.

Experimental –C-

Cytotoxicity :-

The cytotoxicity were tested of (DMPMC) against Red Blood Cell (R.B.C) for human by using (Nair *et al* method)[16]in which 38ml of Ringer solution were mixed with 2ml of blood containing potassium ethylene diammine tetraacetate , from this mixture 2ml were put in each five

sterilized test tubes which contain different concentrations of (DMPMC) (0.1, 0.05, 0.04, 0.03, 0.02M) respectively. After incubation at 37C° for 8 hours , the test tubes were examined at each hour if there is any hemolytic for the (R.B.C) and the result were recorded.



RESULTS AND DISCUSSION

The IR spectrum for (DMPMC) displayed common features in certain regions and characteristic bands in the fingerprint and other regions. The IR spectrum confirm the presence of the N-H stretching with a sharp band at 3267 Cm^{-1}, and C-H stretching of aliphatic methyl groups at 2918 Cm^{-1}as medium band. Furthermore, the IR spectrum is confirmed by the lack of C-H aromatic of (C-4) stretching band in mercurated compound. This conclusion is also supported by ^{1}H-NMR data, which show only two expected peaks in proper intensity ratio one singlet at 2.66 ppm which attributed to protons of two methyl groups and the second singlet at 11.75 ppm attributed to N-H proton . Carbon-13 NMR spectrum gave further support to the formulation of this new compound the ^{13}C-NMR 14.3 ppm (methyl groups), 146.8 ppm (C-3 and C-5), 108 PPM (C-4) the large variation for carbon atom bearing mercury may be attributed to the polarity of C-Hg bond[17].

It is well known that 3,5-dimethyl-1H-pyrazole present as trimer through double hydrogen bond[18] , the (DMPMC) has in addition to the ability of hydrogen bond formation it has mercury atom which can form complexes with essential amino acids through available vacant outer orbital . Generally the data given in table (1) confirm very high inhibitory activity of (DMPMC) at 0.22M.

The disc agar method was also used to evaluate the sensitivity of several species and all the species tested demonstrated zones of inhibition ranged from (10- 35) mm . According to table(1), the minimal inhibitory concentrations level of (DMPMC) against *Esherishia coli, Klebsilla aerogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Candida albicans, A. flavus,A. fumigatus* and *A. niger* were (0.02, 0.02, 0.03, 0.02, 0.02, 0.02, 0.02, 0.02 and 0.02M) and the inhibition zones were (18, 20, 20, 15, 15, 20, 15, 10, and 15)mm in diameter respectively Fig.(1,2). The inhibition zones of *Esherishia coli, Klebsilla aerogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Candida albicans, A. flavus, A.*

fumigatus and *A. niger* were (30, 35, 30, 30, 25, 25, 30, 20, and 25) mm in diameter at 0.22M concentration of (DMPMC) Fig.(1,2). The mechanism of antimicrobial action of (DMPMC) was unknown, but one can suggest that the (DMPMC) at bacteristatic, bactericidal fungistatic and fungicidal level disrupt cell metabolism by binding through hydrogen bond with amino acids and proteins including enzymes or by coordination of amino acids and proteins including enzymes with mercury atom through available vacant orbitals present on it, also may be by destruction of the bacterial cell wall[13] .

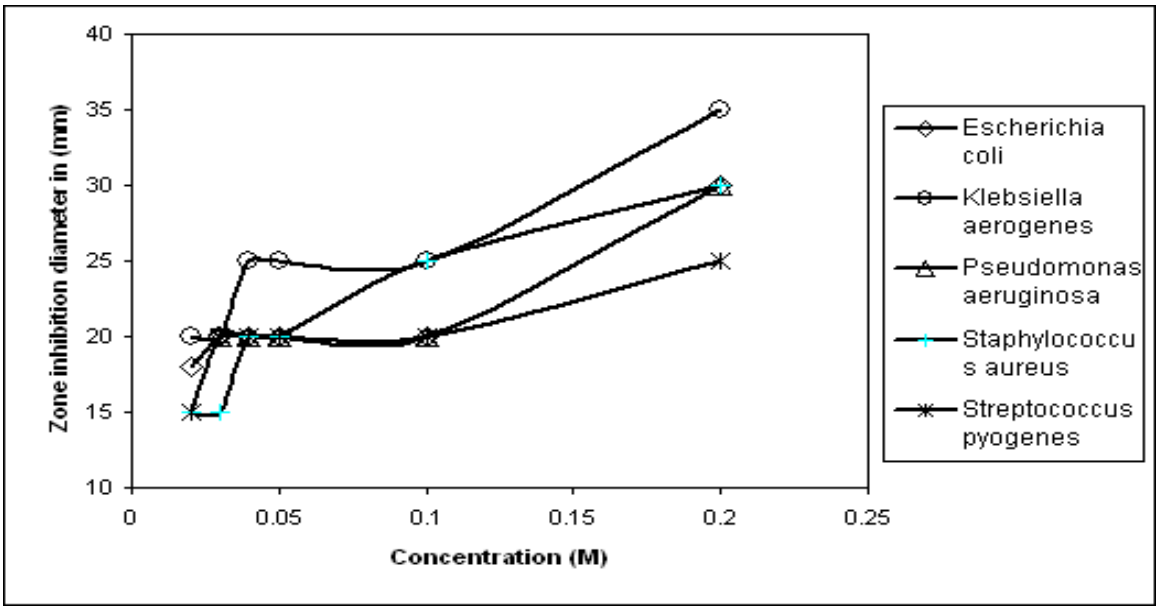
The results shows different inhibitory effect of (DMPMC) on bacterial and fungal isolates , the most gram negative bacterial isolate affected at low concentration (0.02M) is *Klebsiella aerogenes* with inhibition zone (20mm),Fig(4-c), then *Escherishia coli* with inhibition zone (18mm) at the same concentration Fig.(4-B). For *Pseudomonas aeruginosa* was unaffected at 0.02M concentration Fig.(4-D) , but it shows constant inhibition zones at different concentrations (0. 1 , 0.05, 0.04, 0.03M) equal to (20mm). For gram positive bacteria isolates *Staphylococcus aureus* and *Streptococcus pyogene* , the inhibition zone at low concentration (0.02M) is (15mm) Fig (4-E,4-F), that because some bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Staphylococcus aureus* were characterized by it's resistant to different kinds of antibiotics and prevent those antibiotics to arrive to the inhibitory concentration of the cell by complexes essentially present on the external cell wall[19]. The (DMPMC) show high ability in inhibited the yeast (*Candida albican*) at different concentrations and the inhibition zone at all concentrations is (20mm) except at standard concentration (0.22M) which is (25mm) Fig.(4-A).

Ghannoum[20] suggested that the inhibition of lipid biosynthesis leading to cell wall damage in case of *Candida albicans* Fig.(2). The (DMPMC) show also high ability in inhibited the three fungal isolates , the inhibition zones for *A.flavus* and *A.niger* at (0.02M) concentration are (15mm) while the inhibition zone fo *A. fumigatus* at the same concentration is (10mm).see table-1- and Fig-3- (A,B,and C). he mechanisn of antifungal action of (DMPMC) was unknown , but one can suggest that (DMPMC) may be useful in controlling the disease under field conditions [21], that might damage the cell wall of Fungi [22].

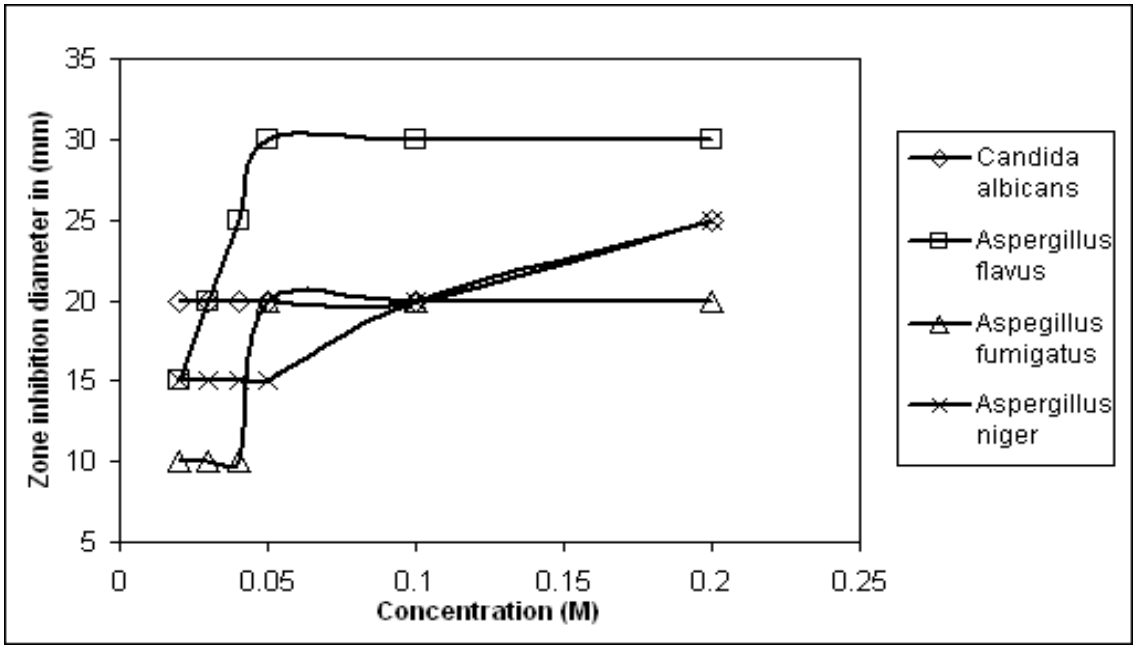
The result of cytotoxicity test against red blood cell for human being shows that the (DMPMC) has no hemolytic at different concentrations and there is no hemolytic for the red blood cell during experimental period (8hours). This result is the first step to continue work with this new compound to produce new drug which may be used in future.

Table-1 :- The inhibition zones in mm diameter for bacterial, yeast and fungal isolates against (DMPMC) at different concentrations

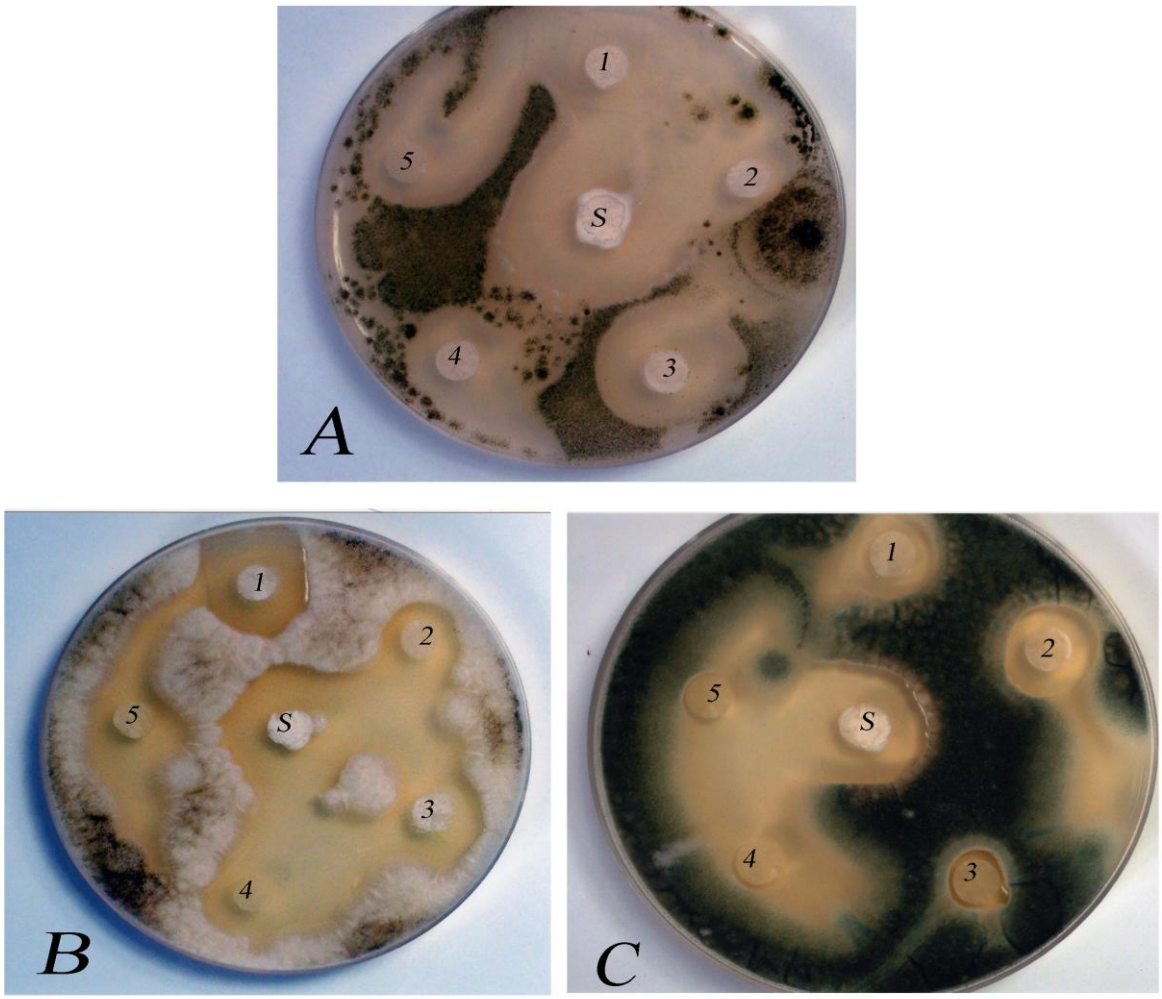
Isolates	Standard 0.22M	0.1M	0.05M	0.04M	0.03M	0.02M
Escherichia coli	**30**	**20**	**20**	**20**	**20**	**18**
Klebsiella aerogenes	**35**	**25**	**25**	**25**	**20**	**20**
Pseudomonas aeruginosa	**30**	**20**	**20**	**20**	**20**	**--**
Staphylococcus aureus	**30**	**25**	**20**	**20**	**15**	**15**
Streptococcus pyogenes	**25**	**20**	**20**	**20**	**20**	**15**
Candida albican	**25**	**20**	**20**	**20**	**20**	**20**
Aspergillus flavus	**30**	**30**	**30**	**25**	**20**	**15**
Aspergillus fumigatus	**20**	**20**	**20**	**10**	**10**	**10**
Aspergillus niger	**25**	**20**	**15**	**15**	**15**	**15**



Fig,(1):- The effect of different concentrations of (DMPMC) in to (MHA) on the growth of Bacteria.



Fig,(2):- The effect of different concentrations of (DMPMC) in to (SDA) on the growth of Fungi.



(Fig3) The sensitivety test of fungi against (3,5 dimethyl-1H- pyrazol-4-yl) mercry (II)chloride by using paper disc method after 24hr from fungi growth at different concentrations.

A:-*Aspergillus flavus*
B:- *Aspergillus niger*
C:- *Aspergillus fumigatus*

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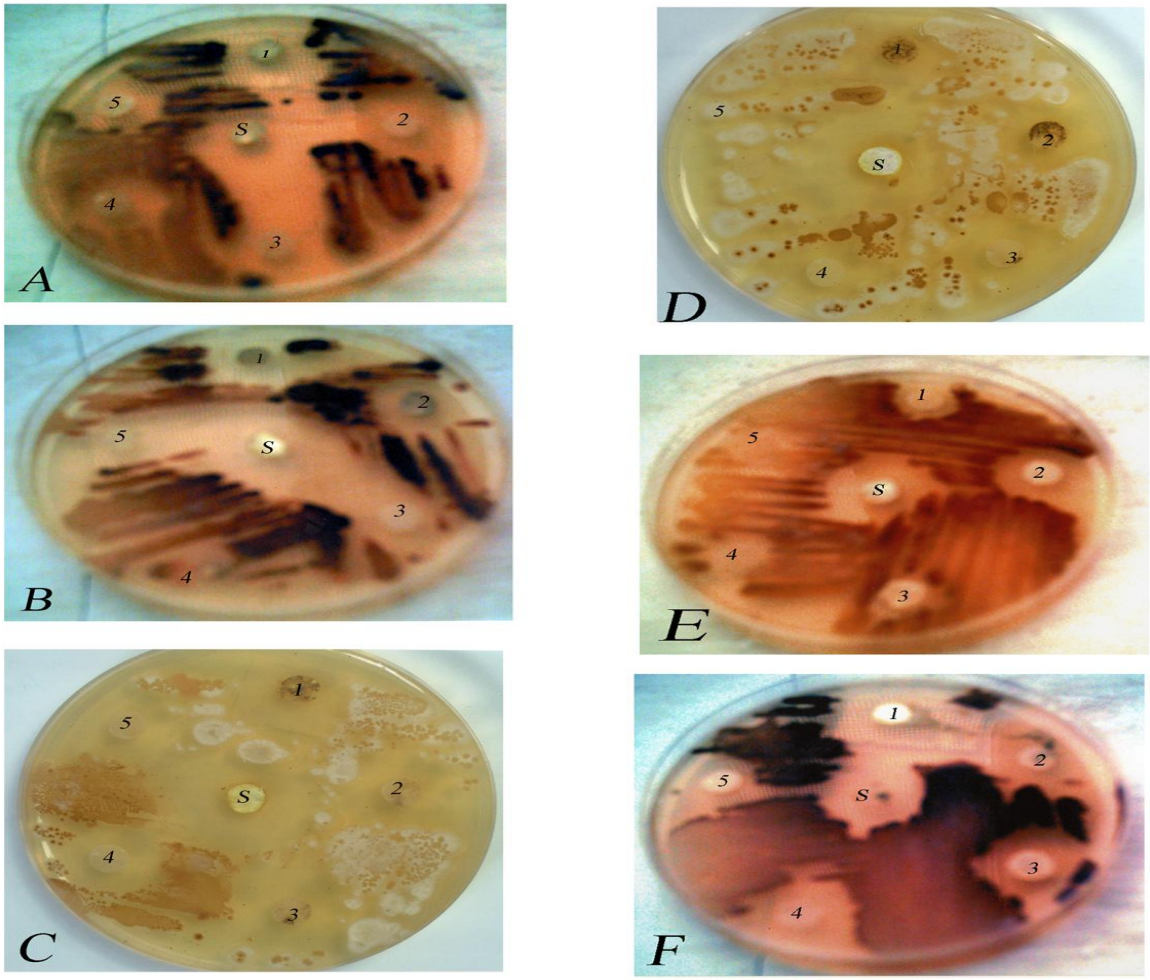

Fig(4)The sensitivety test of Gram-positive and Gram- negative bacteria against (3,5 dimthyl-1H- pyrazol-4-yl) mercury(II) chloride by using paper disc method after 24hr from bacterial growth at different concentrations.

A:-*Candida albicans*
B:- *E.coli*
C:- *Klebsiella aero genes*
D:- *Pseudomonas aeruginosa*
E:- *S..aureus*
F:- S*trept.pyogene*

تحضير وتشخيص ودراسة الأثير المثبط للمركب 5,3-ثنائي مثيل -H1-بايروزول-4-يل) كلوريد الزئبق على نمو بعض البكتيريا والخمائر وبعض الفطريات.

أيمان طارق علي
فرع الأدوية والسموم والعلوم ألمختبريه، كلية الصيدلة ،جامعة البصرة ،البصرة،العراق.

الخلاصة

تضمن البحث زئبقية المركب 5,3- ثنائي مثيل بايروزول بواسطة خلات الزئبق وتم تشخيص المركب باستخدام مطيافية الأشعة تحت الحمراء وطيف الرنين النووي المغناطيسي للبروتون وطيف الرنين النووي المغناطيسي للكاربون-13 بالأضافه إلى التحليل الدقيق للعناصر والتي أكدت صحة الصيغة المقترحة للمركب. حيث وجد إن 0.22M من المركب

(DMPMC) المذاب في مزيج من الأيثانول والماء ثبط نمو البكتيريا سالبة الغرام(*E.coli, Pseudomonas aeruginosa* *Klebsiella aerogene*) والبكتيريا موجبة الغرام (*Staphy.aureus Strept.pyogenes*) والخميرة *Candida albicans* بالأضافه إلى تثبيط نمو بعض الفطريات *-Aspergillus flaves, Aspergillus niger, Aspergillus fumigatus* كما وجد إن التراكيز العالية من المركب (DMPMC) تؤدي إلى تثبيط النمو بشكل اكبر. تمت دراسة التركيز الأدنى المثبط وكذلك دراسة السمية لدم الإنسان ووجد انه لايوجد تحلل لكريات الدم الحمراء عند تراكيز مختلفة من المركب . وتعتبر هذه الدراسة هي الخطوة الأولى لدراسة هذا المركب الجديد والذي من الممكن الأستفاده منه كدواء في المستقبل.

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