Syntheses,Spectrophotometric determination of mercury(II) using new azo dye 5-[(2-hydroxy phenyl azo]-4,6-dihydroxy-2-mercapto pyrimidine.

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Abstract:
New reagent and complex of general formula ML_2,where M=Hg(II),L=5-[(hydroxyl phenyl)azo]-4,6-di hydroxyl-2-mercapto primidine) have been prepared in ethanolic solution. Solid compounds were isolated and characterized by electrons and vibrational spectra. A sensitive method has been developed for the determination of micro amounts of Hg(II).
The method is based on the chelation of metal ion with the azo dye to form an intens color soluble product, that are stable and has a amaximum absorption at (524 nm),and ε_{max} of($4.01x10^4$).The stability constant and relative errors and a relative standard deviations for Hg(II) were($0.14x10^8$),(%-1.1),(%1.42) respectively. The above newly synthesized compounds were investigated for their antibacterial.

ألخلاصة:
تضمن البحث تحضير كاشف عضوي جديد ومعقد في محاليل ايثانوليه صيغته ML_2 حيث ان$M=Hg^{+2}$,وL=5-[(2-هيدروكسي فنيل)ازو]-4,6-ثنائي هيدروكسي -2-مركبتو بريميدين.تم تشخيص الكاشف ومعقده في الحيث ان اله الصلبه بوساطة تحاليل الاطياف الالكترونيه والاهتزازيه. وكذلك تم تطوير طريقه تحليليه سريعه وحساسه لتقدير ايونات الزئبق الثنائية مع الكاشف العضوي الجديد,لتنتج صبغه ارجوانيه مستقره وذائبه في الماء وتعطي اعلى امتصاص عند طول موجي 524 نانوميتر.وكانت قيمة معامل الامتصاص المولاري مساويه إلى (10^4x4.01)لتر. مول$^{-1}$.سم$^{-1}$.وقيمة حساسية ساندل (10^{-3}x5) مايكروغرام.سم^2. والخطأ النسبي (-2 %)-(1.1- %)مع انحراف قياسي نسبي (% 1.42)اعتمادا على مستوى التركيز المراد تحديده .تمت دراسة الفعاليه البايلوجيه للمركبات المحضره كمضادت بكتيريه

key words: 5-[(2-hydroxy phenyl)azo-4,6-dihydroxy-2-mercapto pyrimidine (HPDMP),mercury,spectrophotometry.

Introduction:

In a search of new sensitive and selective reagents, studies of some azo compounds containing various hetro cyclic have been made.[1-6]
Of these QAI 2-(8-Quinoly azo)-4,5-diphenyl imidazole,1-(2-pyridyle azo)-2-naphthol.[7,8] The importance of this element lies in its toxic nature. The biochemical toxicology of the different compounds varies greatly with the chemical form and entrance route in to the body. It has been estimated from animal experiments that (60-90)% of mercury vapour inhaled is taken up by the animal body[9] .The main reasons for the presence of mercury in food come from the residue directly emitted in to the atmosphere from mining operations, coal combustion volcanic activity etc. It was shown recently that mercury(II) could be determination by potentionmetric stripping analysis[10,11].Many analytical techniques are available for the determination of these inorganic substances in industrial and food chemistry; namely cold vapour atomic absorption, neutron activation with radio chemical separation neutron and graphite furnace atomic absorption spectrophotometry.[12] Also earlier studies, titration techniques for penicillin and 6-amino penicillanic acid with mercury(II)solutions were reported. These methods were based on volumetric titration of penicillins hydrolyzed to the corresponding penicilloates with mercury(II) nitrate solution .[13-15] A recent approach has been to use macro cyclic crown ethers or macrobicyclic cryptands compounds as ligands in solvents extraction. Crown ethers show a remarkable ability to selectively extract alkaili and alkaline earth metal ions and also Hg^{+2} [16-18].
The objective of the investigation reported in this paper was to prepare a new azo reagent compound and evaluate aspectrophotometric methods for the determination of Hg(II) based on the

reaction of this ion with new azo reagent 5-[(hydroxyl phenyl)azo]-4,6-dihydroxy-2-mercapto primidine) .A stable soluble-purple color product was formed which can be measured at(524) nm.

Prepare anew azo reagent compound and Experimental Section:-

Apparatus:-

All spectral and absorbance measurements were carried out on a Shimadzu UV-Visible 1700 double beam spectrometer using 1 cm glass cells. Vibration spectra were recorded on test scan Shimadzu FT IR 8000series .A digital PH meter was used

Reagents:-

All the chemicals used were of analytical reagent (AR) grade .Distilled water was used throughout the present study .

— 5-[(hydroxyl phenyl)azo]-4,6-dihydroxy-2-mercapto primidine) ($1x10^{-3}$)M

(0.066)gm of reagent was dissolve in 250 ml of ethanol. Working of (HPDMP)solution was prepared by simple dilution of the appropriate volume of the (HPDMP)solution ($1x10^{-4}$)M with ethanol.

— Standard Hg(II) solution (1000)ppm.

This solution was prepared by dissolving (0.27)gm of $HgCl_2$ in 100 ml distilled water ,working standard of Hg(II)solutions were prepared by simple dilution of the appropriate volume of the standard Hg(II) solution (1000)ppm with distilled water.

— Foreign ions solutions ($1mg.ml^{-1}$)

These solutions were prepared by dissolving an amount of compound in distilled water completing the volume in a volumetric flask.

— Preparation of reagents(HPDMP)

The reagents was prepared by coupling (4,6-dihydroxy-2-mercapto primidine) with appropriate diaztate in alkaline solution. A diazonium solution was prepared by taking (2.0)gm 2-amino phenol in(2)ml of concentrated HCl and (10)ml of distilled water, and adding ($NaNO_2$) drop wise at (0-5)^oc.

4,6-dihydroxyl-2-mercapto primidine(1.5)gm was dissolved in (150)ml of ethanol and (50)ml of NaOH at(-5)^oC .The mixture was allowed to stand over night .The precipitate was filtered off, and recrystallized from ethanol.

Procedure of analytical study

In to a series of (5)ml calibration flask, transfer increasing volumes of Hg(II) working solution (10µg/ml)to cover the range of the calibration curve ,add(1.5)ml of ($1x10^{-4}$)M of (HPDMP) solution and the PH was adjusted by (0.05)N of HCl and (0.05)N of NaOH.

The complex formed was solubilised in ethanol and diluted up to 5 ml with distilled water .scheme(1). And allow the reaction mixture to stand for (5)min at room temperature .Measure the absorbance at (524)nm for Hg(II) complex against a reagent blank prepared in the same way but containing no Hg(II).The color of the formed complex is stable for 24h.

Preparation of complex [$Hg(HPDMP)_2$]

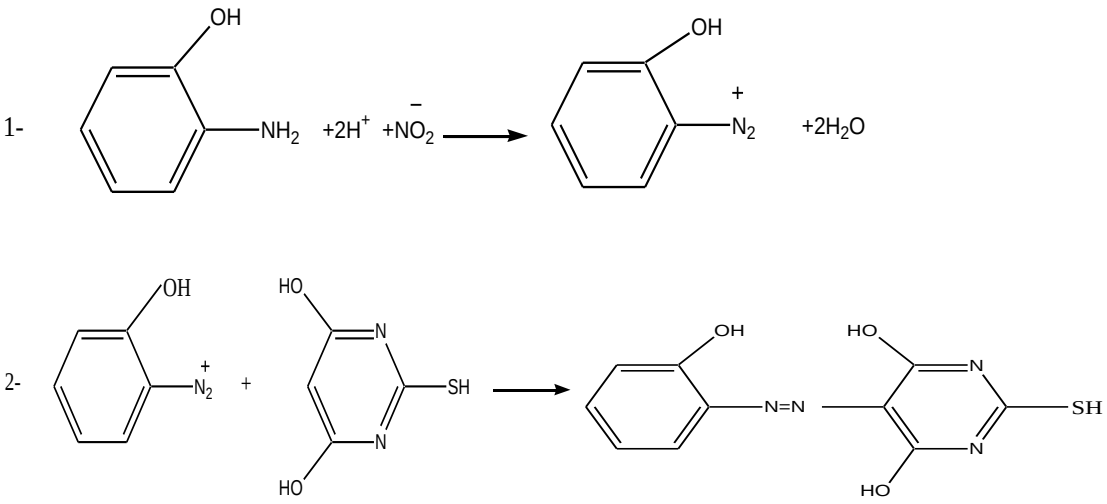
The complex was prepared by mixing stoichiometric amount of $HgCl_2$ and ligand (HPDMP) in a1:2 ratio in aqueous ethanolic solution .The mixture was stirred at room temperature for 3 min .The PH of solution was adjusted to (6) then the solution left at room temperature for 24h.The solution was filtrate by filter paper and washed with distilled water and dried at (80)^oC.

Results and discussion

The mechanism of reagent reaction:

The reaction sequence in procedure of reagent involves two steps. In the first,2-amino phenol reacts with nitrite to form diazonium ion the second including the diazonium ion is coupled with 4,6-dihydroxy-2-mercapto primidine to form yellow azo dye .

This reaction involves the electrophilic substation of diazonium cation at position (5)of 4,6-dihydroxy-2-mercapto primidine and it can be represented as flows:-

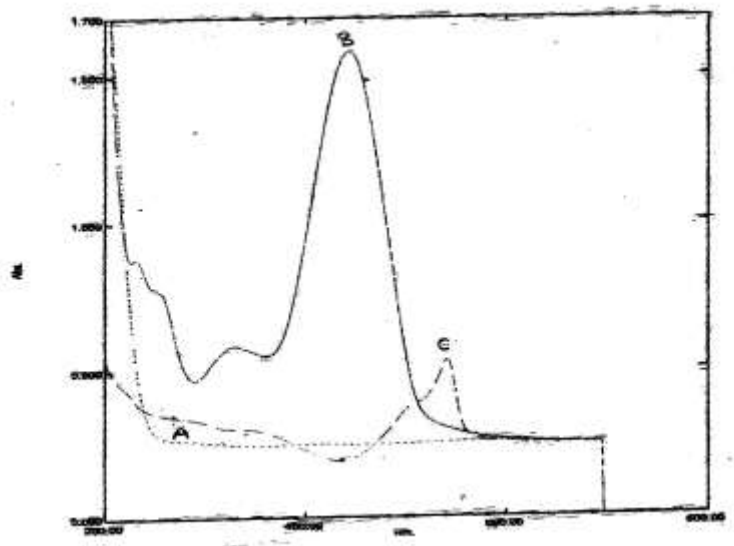


Scheme(1) proposed mechanism of the reaction

Absorption spectra :-

The results of this investigation indicated that the reaction of Hg(II) with (HPDMP)yield highly soluble colored complex which can be utilized as as a suitable assay procedure for determination of Hg(II) .This colored complex has a maximum absorption at (524)nm. The blank at this wavelength shows zero absorbance. Fig(1).

The effect of various parameters on the absorption intensity of the formed products were studied and the reaction condition were optimized.



Fig(1).Absorption spectra of C(10µg/ml)of Hg(II) ion treated as described under procedure and measured against a reagent blank and B the reagent blank measured against distilled water and C the metal ion measured against distilled water.

Effect of solvents on the electronic absorption spectra

The electronic absorption in presence of different solvents gave main points , one of these the position of azo group in the 5-[(hydroxyl phenyl)azo]-4,6-dihydroxy-2-mercapto primidine) is verified to exist in the wavelength range (520-524)nm. The second point intra molecular(H—bond) is detected in all the organic compounds from the blue-shift observed in λ_{max} on changing the solvent from ethanol to water [19].

Also the(n—□*)and (□—□*) electronic transition are identified where in parent phenolic compound ,the bands observed in the range (260-280)nm in different solvents are due to(□—□*) transition of phenyl ring , where those observed in the range (280-330)nm are due to(□—□*) transition .For the 2-hydroxy phenyl azo the bands observed in the range (354-440)nm are due to(n—□*)transitions .Fig (2)

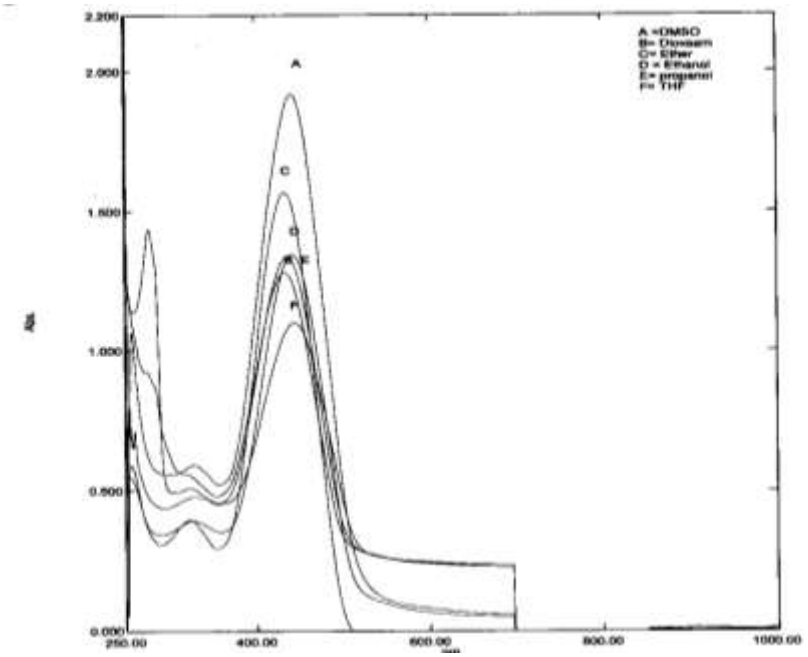


Fig (2):-Absorption spectra of reagent at different solvents

Effect of PH:-

The PH of metal complex solutions was adjusted using dilute solution of (0.05)N HCl and (0.05)N of NaOH, and the effect on absorbance was studied. The absorbance of the complex was maximum and constant in the PH range given in table (1):-

Table -1- Analytical Characteristics of Metal (HPDMP) complex

Characteristic	Hg(II)
Beer's law range (µg/ml)	0.1—1.3
Absorption maxima (λ max , nm)	524
pH range	5.5—7
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	$4.01x10^{4}$
Sand ell's sensitivity (µg/ cm^2)	$5x10^{-3}$
Stability constant ($L^2.mol^{-2}$)	$0.14x10^{8}$

Effect of reagent concentration

When various concentration of 5-[(hydroxy phenyl)azo]-4,6-dihydroxy-2-mercapto primidine) solutions were added to affixed amount of Hg(II) ,(1.5)ml of ($1x10^{-4}$)M reagent was found enough to develop the color to its full intensity and give a minimum blank value and was considered to be optimum for the concentration (0.1—1.3µg/ml)of Hg(II) .

Calibration curve:-

The calibration curve was constructed at their respective absorption maxima and these were linear over concentration range as given in Table(1) ,for metal ion .The molar absorpitivity and Sandall's sensitivity are given in table (1)

Development time and stability period

The color intensity reached maximum after metal ion had been reacted with (HPDMP).The color obtained was stable for at least(24h) and this period was sufficient at allow several measurements to be performed sequentially

Composition of the complex

The composition of the complex was studied by Job's method[20].

A break at a (1:2) (M:L) suggested the formation of $M(HPDMP)_2$ where M=Hg(II) Fig(3).

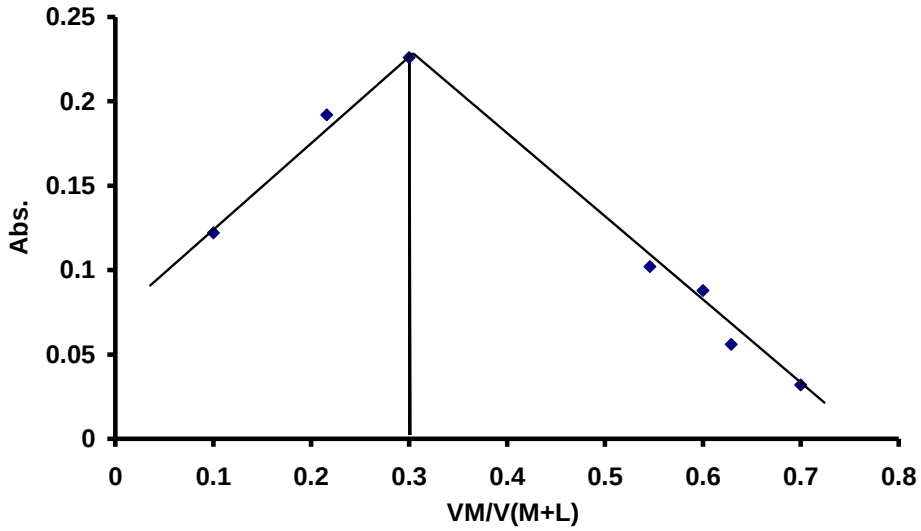


Fig (3) study of the mole ratio of the reaction between (HPDMP)and Hg(II)
(Job's method)

Sensitivity :-

To determine the accuracy and precision of the method ,mercury ion was determined at two different concentration .The results shown in table (2), indicate a satis factory precision and could be obtained with proposed method.

Table (2):-Accuracy and precision of the method

Amount of Hg(II) taken ppm	Error%	R.S.D%
1	-1.1	1.42
0.5	-2	5.3

Results for five determination.

Interferences

The effects of diverse ions on determination of this metal ion was studied. To test of diverse was ions were determined by the general procedure, in the presence of their respective foreign ions. The metal ion can not be determined in the presence of a 10 or more fold excess of cat ion table(3)

Table (3):-Effect of foreign ions

Foreign ions	Amount added (µg)	Interference
NO^{-}_{3}	1 00	%7.7
Ni^{+2}	=	%31.1
Co^{+2}	=	%52.4
Cr^{+3}	=	%14.7
Cl^{-}	`=	%1.2
CO^{-2}_{3}	=	%7.3
SO^{-2}_{4}	=	%-0.4
Zn^{+2}	=	%31.1
Cd^{+2}	=	%22.9
Pt^{+2}	=	%31.5
Fe^{+3}	=	%47.5
Pd^{+2}	=	%78.6
Cu^{+2}	=	%71.7
Rh^{+2}	=	%54.5
Ag^{+}	=	%91.8
NO^{-}_{2}	=	%4.9

Infra red spectra of the reagent and the complex

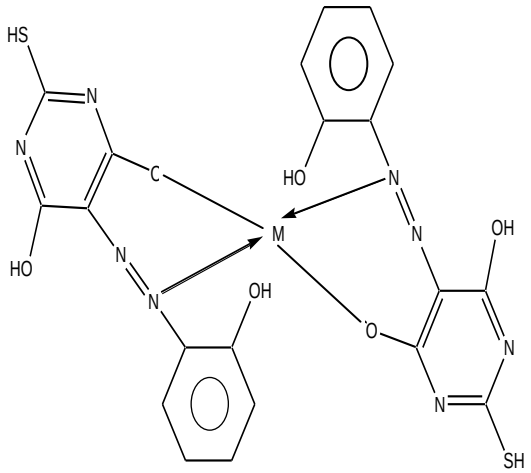
All the spectra were recorded in the solid state using FTIR in the range (500-4000 cm^{-1}).The I.R. bands of the (HPDMP)and its mercury complex with their probable assignment are given in table (4)Fig (4,5)

Table (4):-Selected I.R. bands of (HPDMP) and it Hg(II) complex

Vibrations	Ligand (HPDMP)	Hg(II)
υ O—H	3380 M	3400 W
υ C—H (Aromatic)	3105 W	3100 W
υ C= N	1645 S 1610 M	1620 W 1580 W
υ N= N	1465 S 1360 W	1445 M
υ S—H	2550 v W	2560 v W
δ N—H	1430 S	1440
Thio amide(I) = = (II) = = (III)	1545 W 1270 M 970 W	1545 W 1340 M 960 W
υ M—O	—	510 W
υ M—N	—	550 W

S=strong M=medium W=weak V W=very weak

On the basis of I.R. stoichiometric data the structure of complex can be suggested as follows:-



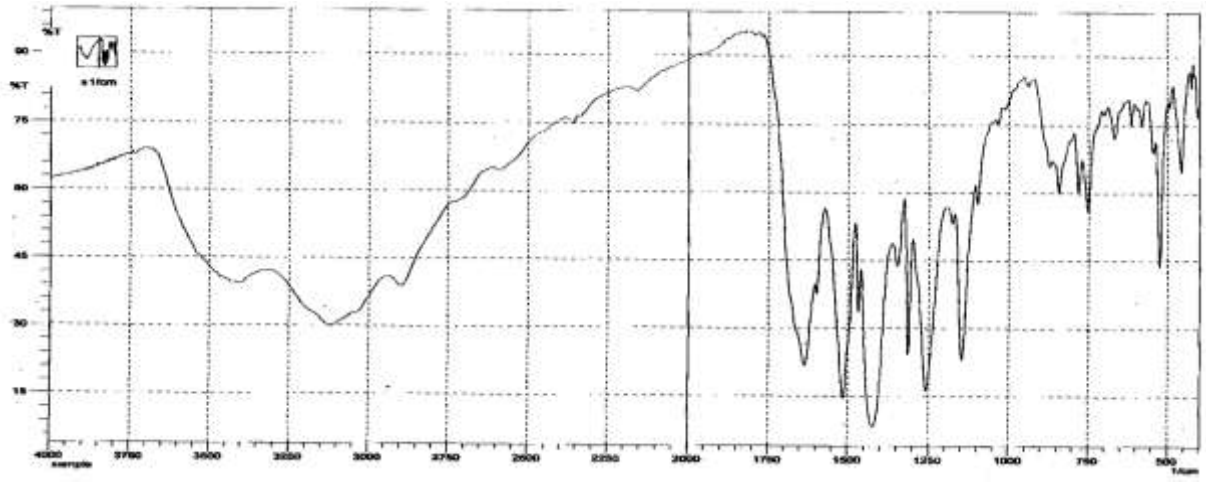


Fig (4) I.R. spectrum of the reagent (HPDMP)

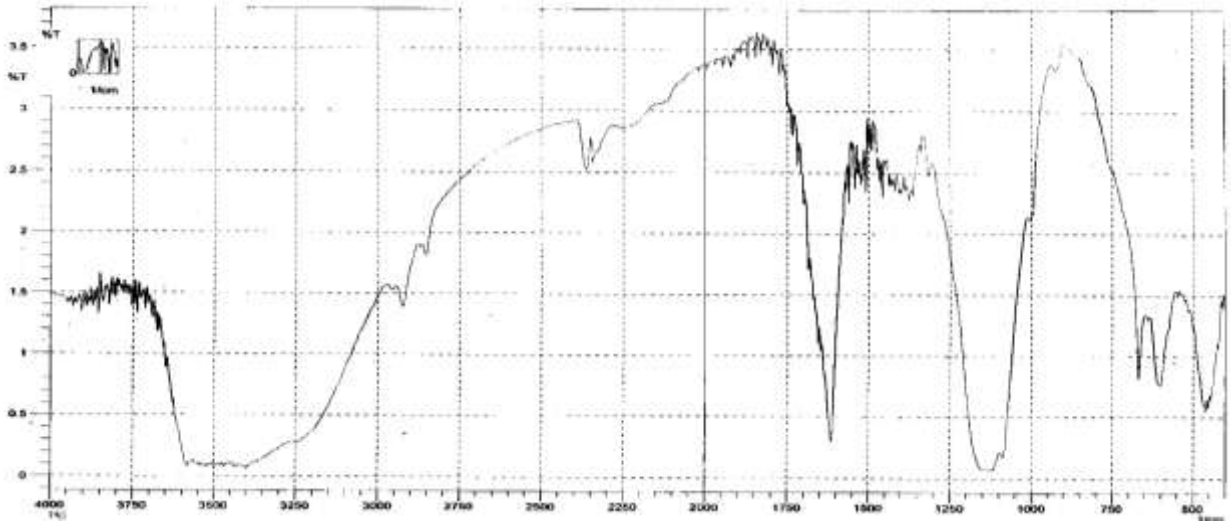


Fig (5) I.R. Spectrum of the complex of Hg^{+2} with (HPDMP)

Biological effect

Agar diffusion method [21] ,was used for the determination of antibacterial activity of the prepared compounds. 0.1 ml of an over night broth bacterial culture was spread on anutrient agar. Sterilized discs (6mm in diameter).Evolution of the above mentioned compounds for their antimicrobial activities showed that these compounds exhibited both antibacterial actives. The results are presented in table(5) Fig(6) .The tested complex showed activity against staphylococcus aureus and pseomonus aeroginosa.Where as compound (HPDMP) showed no activity against all types of bacteria. It should be mentioned that the antimicrobial results were obtained concentration ($1x10^{-3}$)M for all tested compounds.

Table(5):-Antibacterial activities of the tested synthesized compounds

Comp. $(1x10^{-3})M$	E.Coli	Staphylococcus aureus	Pseudomonas aerginose
HPDMP	—	—	—
$Hg(HPDMP)_2$	—	+	++

— =no inhibition + = (7-10) mm ++ = (10-12) mm

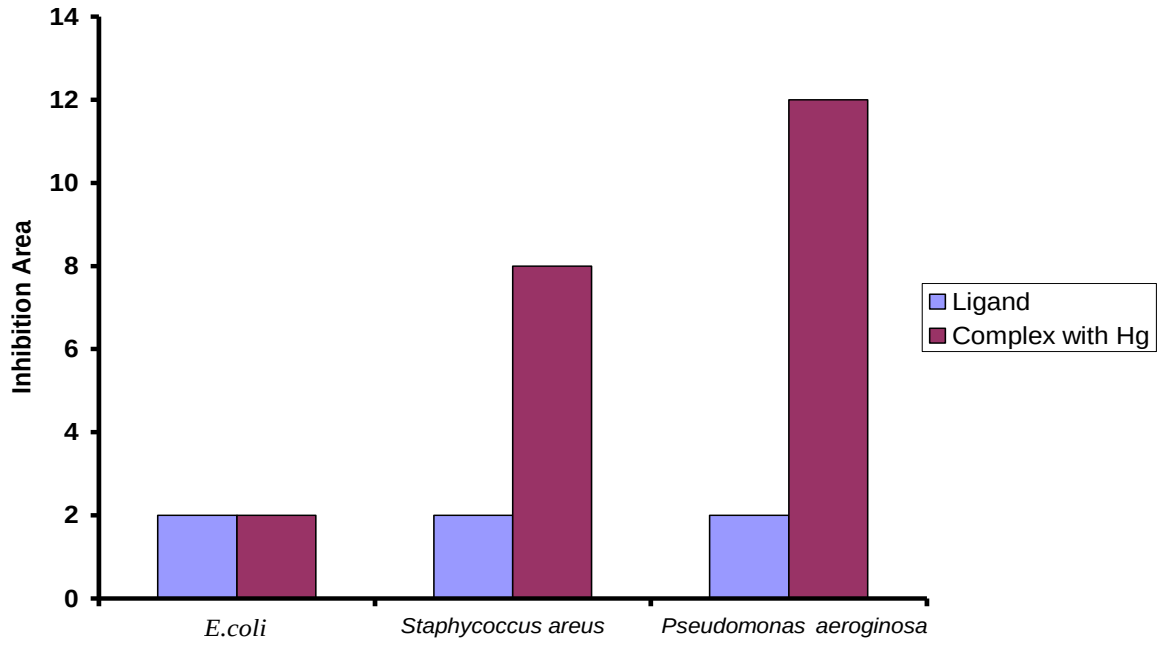


Fig (6):-Inhibition zones of the reagent (HPDMP) and its complex

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