



Research Article

## Spectrophotometric Determination of Cobalt(II) and Lead(II) Using (1,5-Dimethyl-2-Phenyl-4-((2,3,4-Trihydroxy Phenyl) Diazenyl)-1H-Pyrazol-3(2H)-One) as Organic Reagent: Using It as Antimicrobial and Antioxidants

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### Abstract

The azo organic reagent (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) (DTPD) was prepared and used for the spectrophotometric determination of cobalt(II) and Lead(II), by the selective and surfactant-sensitized method based on the ternary complexes formation of Co(II) and Pb(II). The reagent had absorption maximum at 381 nm, and reacted with  $\text{Co}^{2+}$  to form a purple reddish complex with  $\lambda_{\text{max}} = 430$  nm at pH = 7.5, while it formed a red complex with  $\text{Pb}^{2+}$  of  $\lambda_{\text{max}} = 417$  nm at pH = 6. Beer's law for the determination over the range of 1-25 ppm and 1-33 ppm for Co(II) and Pb(II), respectively. The molar absorptivity ( $\epsilon$ ) and Sandell's sensitivity values of Co(II) and Pb(II) complexes were found to be  $1.02 \times 10^4$ ,  $3.3 \times 10^4$   $\text{mol}^{-1} \text{cm}^{-1}$ , and 0.0725,  $0.0269 \mu\text{g cm}^{-2}$  at 430, 417 nm, respectively. The stability constant was found to be  $1.1 \times 10^8$   $\text{L mol}^{-1}$  and  $2.3 \times 10^8$   $\text{L mol}^{-1}$  for Co(II) and Pb(II), respectively. Detection limit relative standard deviation, relative error and recovery were predestined for 15 ppm standard solution of Co(II) and Pb(II) complexes, respectively. The important interferences with most ions like  $\text{Cr}^{+3}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Mo}^{+2}$ ,  $\text{Pt}^{2+}$  and  $\text{Cd}^{+2}$  were studied using the appropriate masking agents. The method was applied for the determination of Co(II) in filling sample; the result obtained was incompatible with that by the flame atomic absorption spectrometry method. The organic reagent (DTPD) was diagnosed as an anti-bacterial and an antioxidant.

**Keywords:** Heterocyclic; Pyrazol; Azo; Antipyrene; Antimicrobial; Antioxidants

### Introduction

Antipyretics were synthesized in 1884, and through research and studies this organic compound was discovered to be a pain reliever. By 1930, it had become one of the most important analgesics

in the pharmaceutical world. With time passing by, antipyretics have proven to be of great value among pharmaceuticals [1, 2].

In this study, the heterocyclic azo dyes were synthesized and proposed as a highly sensitive chromogenic reagent for the determination of

several metal ions [3, 4]. The chemical compound (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) was prepared [5] and used as organic reagent and antioxidant compound in this study.

Cobalt is an element of high industrial importance in alloying, coating and dyeing. It is used as a catalyst because of its important properties; it is a transitional element and also has biological usages as this element can be an active center of auxiliary enzymes, for example vitamin B (cyanocobalamin), which is used as radiation therapy for cancer patients [6, 7].

Lead is a major cause of many human killer diseases, leading to dysfunction of blood and nervous system. Lead can also precipitate easily in the brain, kidneys and reproductive system. Another popular disease is lead poisoning, which leads to anemia and brain damage and ultimately to death [8].

Several techniques such as flame atomic absorption, voltammetric and spectrophotometric methods have been used for determination of the ion in different samples, among which ultraviolet-visible spectrophotometry (UV-Vis) is the most commonly used technique for Pb(II) [9].

There are many studies conducted to determine the elements; however, our research for the first time completed the estimation of cobalt(II) and lead(II) using (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) as organic reagent. On the other hand, this work has been applied in 2 ways: The first with regard to the reagent in a field of antioxidant, and the second concerning determination of cobalt in the filling of teeth.

## Experimental

### Apparatus

Absorption spectra in absolute ethanol were recorded using UV-Vis spectrophotometer T80, England, using 1 cm quartz cells. Functional groups of reagent and its complexes were identified using Fourier-transform infrared (FTIR) spectrometer Shimadzu 8400, in the range of 4000-400  $\text{cm}^{-1}$  using KBr disc. pH measurements were carried out using a Philips PW 9421 pH meter ( $\text{pH} \pm 0.001$ ), AA-6300 atomic absorption spectrophotometer, Pg, Japan.

### Reagents and solutions

All chemicals used were of high purity without

additional purification. Ethanol was purchased from GCC, England. 5-sulfosalicylic acid,  $\text{NaNO}_2$ , sodium acetate, oxalic acid, ascorbic acid, tartaric acid,  $\text{Na}_2\text{S}_2\text{O}_3$ , KCl and KI were purchased from Sigma-Aldrich. The reagent  $1 \times 10^{-4} \text{ mol L}^{-1}$  (1,5-dimethyl-2-phenyl-4-((2,3,4-tri-hydroxy phenyl) diazinil)-1H-pyrazol-3 (2H)-one) was prepared for dissolving route of reagent in 250 mL absolute ethanol.

Co(II) and Pb(II) ion solutions ( $1000 \text{ mg L}^{-1}$ ) were prepared by dissolving 0.4033 g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (BDH) and 0.1598 g of  $\text{Pb}(\text{NO}_3)_2$  in 100 mL deionized water, standard solutions for each metal were made by appropriate dilutions.

### Antimicrobial study

In a standard 10 mL conical flask, 1.5 mL of Co(II) solution containing less than  $100 \text{ gm mL}^{-1}$  of it was transferred, where the pH was adjusted to 7.5 using ammonium acetate solution; then 2 mL  $1.0 \times 10^{-4} \text{ M}$  of ethanolic reagent was added and diluted with deionized water to the marker. At 430 nm and  $25^\circ \text{C}$ , the resulting solution absorption was measured after 10 min. In the same way, a solution was prepared for a lead(II) element but with a pH = 6, and the absorbance was measured at 417 nm after 10 min.

The antibacterial tests were performed according to the disc diffusion method. (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) (DPTPD) was examined for its antimicrobial action in vitro against 4 strains of bacteria, 2 of which were gram-positive and 2 gram-negative represented by *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae*, respectively. In addition, single fungal - *Candida albicans* was used; amoxicillin and cephalixin were utilized as a positive control and DMSO was employed as a negative control; nystatin was employed as antifungal reference drug.

Bacteria were incubated and cultured at  $37^\circ \text{C}$  for 24 h. To prepare media, agar was poured into the sterilized petri discs. After that, the cultures were scattered on the surface of nutrient agar (NA). Antimicrobial action was defined by measuring the diameter of inhibition zone (IZ) in comparison with positive control where the concentration of all compounds was  $30 \mu\text{g mL}^{-1}$ .

### Antioxidants study

To clarify whether the reagent (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) had the potential to act as an

antioxidant. The plasmid was first extracted from *E. coli* bacteria according to the extraction method of the kit; then the electrophoresis for the samples was done. The extract was good and gave clear bands.

## Results and Discussion

### Infrared spectra

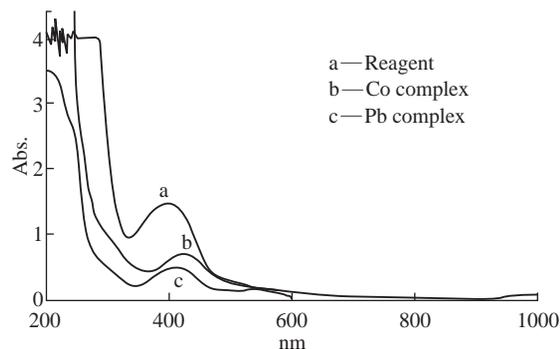
The IR spectrum of the reagent showed absorption at  $1710\text{ cm}^{-1}$  for C=O,  $1504\text{ cm}^{-1}$  for -N=N-,  $3455\text{ cm}^{-1}$  for OH of phenol, and showed band at  $3088\text{ cm}^{-1}$  for aromatic C-H and band at  $2994\text{ cm}^{-1}$  for aliphatic C-H. The presence of new medium intensity bands in the  $420 - 455$  and  $480 - 520\text{ cm}^{-1}$  regions, assignable to  $\nu(\text{M-N})$  and  $\nu(\text{M-O})$  in the spectra of all complexes [10, 11] as shown in Table 1.

**Table 1** Important IR frequencies for the ligand and its complexes

Compound	$\nu(\text{O-H})$	$\nu(\text{N=N})$	$\nu(\text{C=O})$	$\nu(\text{Ar-H})$	$\nu(\text{M-O})$	$\nu(\text{M-N})$
Reagent	3380	1504	1710	3067	--	--
Co-complex	3329	1572	1664	3066	488	455
Pb-complex	3388	1563	1711	3088	501	401

### Absorption spectra and characteristics of the complexes

UV-Vis for the ethanolic solution of the reagent ( $1 \times 10^{-4}\text{ M}$ ) showed 3 clear peaks. The first and the second peaks were at 279 nm and 239 nm and indicated the transition of  $-\pi^*$  of the aromatic rings. The third peak was at 381 nm and attributed to  $\pi-\pi^*$  of the aromatic ring through the azo group. Transfer of charge referred to the transmission of n- of  $\pi^*$  to charge the intermolecular charge place of benzene through the



**Fig. 1** Absorption spectra of (a) reagent =  $1.0 \times 10^{-4}\text{ M}$ , (b) Co(II)-complex (15 ppm) at pH = 7.5, and (c) Pb(II)-complex (15 ppm) at pH=6.

azo group -N=N [12].

In aqueous ethanol solution, the reaction of metal ions Co(II) and Pb(II) with the reagent was studied. A bathochromic shift of Co(II) and Pb(II) complexes showed the absorption maxima of 430 and 417 nm with molar absorptivities ( $\epsilon$ ) as of  $1.02 \times 10^4\text{ L mol}^{-1}\text{ cm}^{-1}$  and  $3.3 \times 10^5\text{ L mol}^{-1}\text{ cm}^{-1}$ , respectively. The reagent gave the absorption maxima of 381 nm as depicted in Fig. 1. The wavelength difference  $\Delta\lambda_{\text{max}}$  was 49-36 nm; a great bathochromic shift in the visible region was detected in the complex solution's spectra concerning that of the free reagent. The high shift in the  $\lambda_{\text{max}}$  gave a good indication for complex formation.

### Method validation

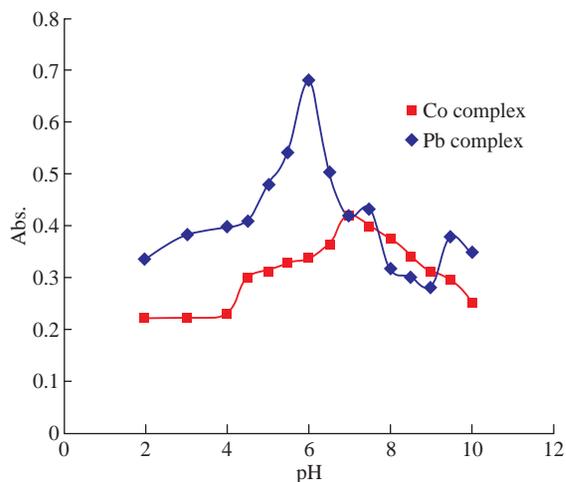
Under optimal conditions, graphs were designed based on optimal calibration conditions and were done by drawing the absorption signal versus the concentrations of each analytical substance. In the 10 mm optical cell, solutions were placed to measure the spectral metal ion at maximum absorption. The calibration data is shown in Table 2.

**Table 2** Method validation of the spectrophotometric determination of Co(II) and Pb(II)

Parameter	Co(II)	Pb(II)
$\lambda_{\text{max}}$ (nm)	430	417
Regression equation	$Y = 0.0138X + 0.0119$	$y = 0.0372x + 0.1115$
Correlation coefficient(r)	0.9975	0.9947
Concentration range ( $\mu\text{g mL}^{-1}$ )	1-25	1-33
Limit of Detection ( $\mu\text{g mL}^{-1}$ )	0.241	0.3
Sandell's sensitivity ( $\mu\text{g cm}^{-2}$ )	0.0725	0.0269
Molar absorptivity ( $\text{L mol}^{-1}\text{ cm}^{-1}$ )	$3.3 \times 10^4$	$1.02 \times 10^4$
Composition of complex (M:L)	1:2	1:2
RSD% (n = 7) at $15\text{ }\mu\text{g Co(II) mL}^{-1}$ and $15\text{ }\mu\text{g Pb (II) mL}^{-1}$	2.1	2.6
Recovery%	99	97.54

### Effect of pH

The effect of pH on the formation of Co(II) and Pb(II) complexes was determined by recording their absorbance signals at  $\lambda_{\max}$ , over the range of 3-9, using different pH acetate buffer solutions. The results are described in Fig. 2.



**Fig. 2** Effect of pH on complexes formed with Co(II) and Pb(II). Conditions: Co(II) =  $15 \mu\text{g mL}^{-1}$ , and 2 mL of the reagent =  $1.0 \times 10^{-4}$  M; Pb(II) =  $15 \mu\text{g mL}^{-1}$ , and 2 mL of the reagent =  $1.0 \times 10^{-4}$  M.

From Fig. 2, it can be seen that the absorption increased with the increase of pH, where absorption increased and reached the maximum at pH 7.5 and 6 for Co(II) and Pb(II) complexes, respectively.

A gradual decrease in absorbance was then observed due to the partial disintegration of complexes at high pH. From all these observations, pH 7.5 and 6 were selected as the best pH for the formation of Co(II) and Pb(II) complexes, respectively.

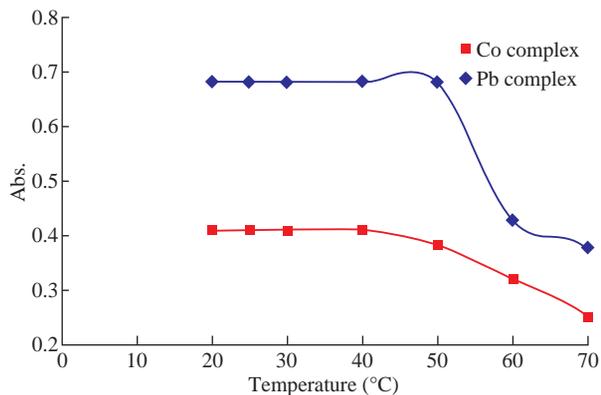
### Effect of temperature and time

The effect of temperature and time was studied for their importance in completing the reaction. Experiments were conducted to determine the temperature range at which the high absorption signals of Co(II) and Pb(II) were apparent. Temperatures between 20 and 70 °C were found to have the optimum absorption value. The best temperature was 40 °C, where the highest absorption signal is shown in Fig. 3.

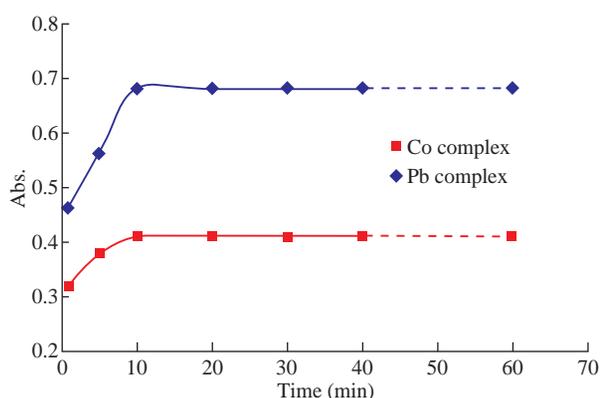
It was also observed that the incubation time of 10 min was sufficient for the maximum absorbance of Co(II) and Pb(II), as shown in Fig. 4.

### Composition and stability of complexes

The composition of chelate complexes was determined by mole ratio and continuous variation



**Fig. 3** Effect of temperature on the absorbance of complexes formed with Co(II) with 2 mL of the reagent =  $1.0 \times 10^{-4}$  M, and Pb(II) =  $15 \mu\text{g mL}^{-1}$  with 2 mL of the reagent =  $1.0 \times 10^{-4}$  M.

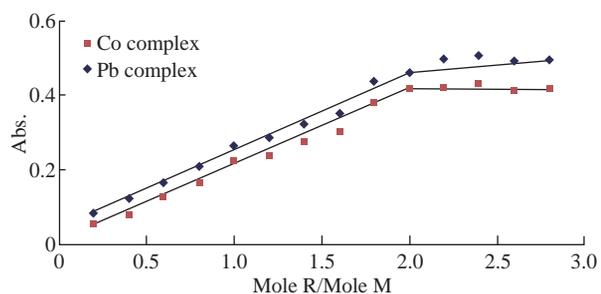


**Fig. 4** Effect of time on the absorbance of complexes formed with Co(II) and Pb(II). Conditions: Co(II) =  $15 \mu\text{g mL}^{-1}$ , 2 mL of the reagent =  $1.0 \times 10^{-4}$  M, and Pb(II) =  $15 \mu\text{g mL}^{-1}$ , 2 mL of the reagent =  $1.0 \times 10^{-4}$  M.

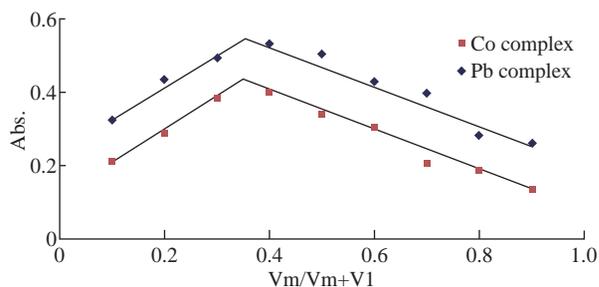
method (Fig. 5 and 6). Both methods showed that the molar ratio of Co(II) and Pb(II) ions to reagent was 1 : 2, and the suggested related chemical structures are shown in Fig. 7. The stability constant was found to be  $1.1 \times 10^8 \text{ L mol}^{-1}$  and  $2.3 \times 10^8 \text{ L mol}^{-1}$  for Co(II) and Pb(II), respectively.

### Interference studies of cobalt(II) and lead(II) complexes

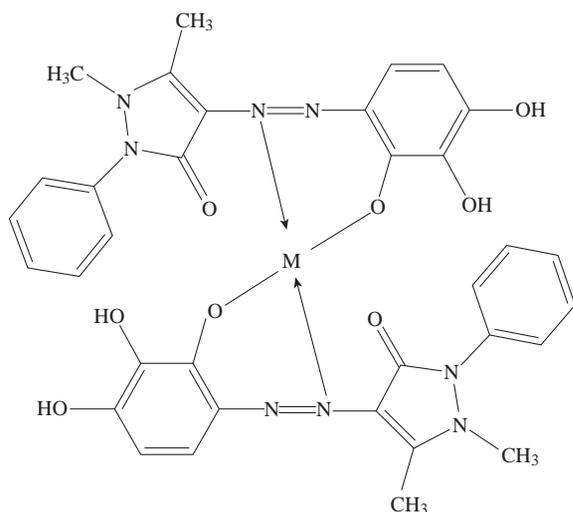
The effect of the interference of ions which formed



**Fig. 5** Mole ratio method for Co(II)-complex at pH = 7.5 and Pb(II)-complex at pH = 6.



**Fig. 6** Continuous variation method for Co(II)-complex at pH = 7.5 and Pb(II)-complex at pH = 6.



**Fig. 7** Proposed structural formula for the complexes (M = Co(II) and Pb(II) ion).

complexes with the reagent (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) during its reaction with cobalt and lead (15 ppm) was studied. The selectivity of the various masking agents was examined for eliminating the effect of each of the interfering 8 ions. These were 5-sulfosalicylic acid,  $\text{NaNO}_2$ , sodium acetate, oxalic acid, ascorbic acid, tartaric acid,  $\text{Na}_2\text{S}_2\text{O}_3$ , KCl and KI. The results are shown in Table 3 and 4.

**Table 3** Effect of exotic ions on the determination of Co(II) and in concentration (15) ppm and suitable masking agents

Foreign ion 100 ppm	Masking agent (1.0) mL [0.01] M	Error%
$\text{Cr}^{+3}$	5-Sulfosalicylic (0.5) [0.01]	0.003
$\text{Mn}^{2+}$	$\text{NaNO}_2$ (0.2) [0.1]	0.002
$\text{Fe}^{3+}$	5-Sulfosalicylic (0.75) [0.01]	0.004
$\text{Zn}^{2+}$	Sodium acetate (0.5) [0.01]	-0.001
$\text{Hg}^2$	Oxalic acid (0.4) [0.2]	-0.002
$\text{Mo}^{+2}$	Oxalic acid (0.4) [0.2]	-0.004
$\text{Pt}^{2+}$	Sodium acetate (0.5) [0.01]	-0.001
$\text{Cd}^{+2}$	5-Sulfosalicylic (0.75) [0.01]	0.001
$\text{Pb}^{+2}$	Ascorbic acid	0.001

**Table 4** Effect of exotic ions on the determination of Pb(II) in concentration 15 ppm and suitable masking agents

Foreign ion 100 ppm	Masking agent (1.0) mL [0.01] M	Error%
$\text{Cr}^{+3}$	KCl (0.5) [0.02]	0.003
$\text{Mn}^{2+}$	Ascorbic acid (0.3) [0.1]	0.002
$\text{Fe}^{3+}$	5-Sulfosalicylic(0.75) [0.01]	0.004
$\text{Zn}^{2+}$	KI (0.4) [0.01]	-0.001
$\text{Hg}^2$	Tartaric acid (0.5) [0.3]	-0.002
$\text{Mo}^{+2}$	Oxalic acid (0.4) [0.2]	-0.004
$\text{Pt}^{2+}$	Sodium acetate (0.5) [0.01]	-0.001
$\text{Cd}^{+2}$	5-Sulfosalicylic (0.75) [0.01]	0.001
$\text{Co}^{+2}$	$\text{Na}_2\text{S}_2\text{O}_3$ (0.05) [0.01]	0.001

## Application

The proposed method was applied to the determination of cobalt in filling. The results are shown in Table 5. The obtained average of the 3 determinations was compared with that given by the atomic absorption spectrophotometer (AAS) (standard addition method).

**Table 5** Determination of cobalt in filling

Standard sample	Certified content (%)	Proposed method (%)*
Rua Funchal 376**	3.30	3.05±0.13

\*Average of 3 determinations, at 95% confidence level.

\*\*Provided from Degussa Dental Ltd., Brasil. Sample Composition Ag (71%), Sn (25.7%), and Co (3.3%).

## Antimicrobial actions

The antibacterial and antifungal actions of (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) (DPTPD) was examined against 2 samples of gram-positive bacteria and 2 samples of gram-negative bacteria, i.e. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae*, respectively, as well as single fungal *Candida albicans* utilizing disc diffusion method. Amoxicillin and cephalixin were used as positive controls; nystatin was used as positive control for fungal (concentration of  $30 \mu\text{g mL}^{-1}$  for all of these compounds). The outcomes are itemized in Table 6 and 7.

The antibacterial activity of (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) was higher than amoxicillin but lowers than cephalixin because (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) contained 4 atoms of nitrogen which

**Table 6** Antimicrobial result for (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) compound

Compounds (30 mg mL <sup>-1</sup> )	Inhibition zone (mm)			
	Gram-positive		Gram-negative	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
DPTPD	17.3	16.7	17	18.7
Amoxicillin	14.4	13.4	15.1	12.9
Cefalexin	18.2	16.3	18.1	18.6
DMSO	--	--	--	--

**Table 7** Inhibition zones of (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) and the references antifungal

Compounds (30 mg mL <sup>-1</sup> )	Inhibition Zone (mm)
	<i>Candida albicans</i>
DPTPD	19.6
Nystatin	17.4
DMSO	--

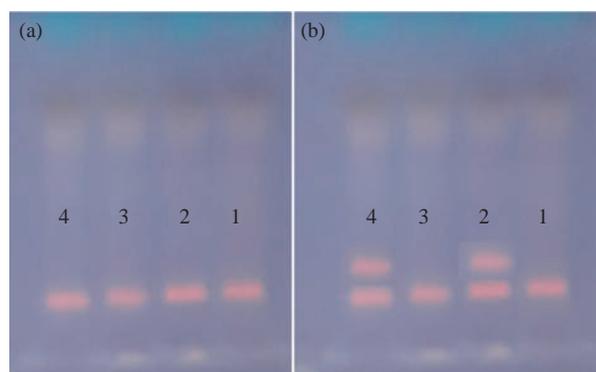
increased the lipophilicity of this compound, while amoxicillin had only 1 atom of sulfur in its structure. These features displayed that the compounds generally enhanced permeability towards gram-positive and gram-negative bacteria cell tissue. It was identified that (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) compound showed good antimicrobial actions in general.

### Antioxidants

The way to know the reagent's ability to act as an antioxidant is to add the Fenton reagent (which contains hydrogen peroxide, a strong oxidizer with catalysts, ferric chloride and ascorbic acid) to the plasmid, where the Fenton compound works on the oxidation of the plasmid, and the plasmid is destroyed. Hence, when the working electrophoresis of the plasmid completed oxidation, it does not give a single band but rather 2 or more bands, or the bands disappear permanently according to the degree of damage.

In our experiment, 2 bands appeared, indicating the plasmid was split into 2 parts due to the oxidizing factors in the Fenton reagent, as shown in Fig. 8 (locations 2 and 4). On the other hand, when adding the compound (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) to tubes 1 and 3, it appeared that the reagent Fenton did not affect the plasmid as the compound acted as an antioxidant, interacted with the Fenton reagent, and canceled its susceptibility to oxidation. Therefore, the single band appeared at sites 1 and 3, which indicated

the plasmid was not affected by the oxidizing agent due to the presence of compound (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) working as antioxidants. These results are consistent with many studies such as those conducted by Razack et al. [13] and by Lahneche et al. [14].



**Fig. 8** (a) Electrophoresis of plasmid extracted from *E. coli*. (b) Plasmid electrophoresis image after plasmid (0.5 µg) was used with Fenton reagent (30 mM H<sub>2</sub>O<sub>2</sub>, 50 µM ascorbic acid, and 80 µM FeCl<sub>3</sub>). The compound (1,5-dimethyl-2-phenyl-4-((2,3,4-trihydroxy phenyl) diazenyl)-1H-pyrazol-3(2H)-one) added to model No. 1 and No. 3.

### Conclusions

Synthesis and preparation of organic reagent were widely used in this study. We found that the reagent (1,5-dimethyl-2-phenyl-4-((2,3,4-tri-hydroxy phenyl) diazinil)-1H-pyrazol-3(2H)-one) reacted with elemental solutions of Co(II) and Pb(II), and that insoluble complexes were formed in water. The spectrum method was found to be simple, fast and uncostly, providing accurate results compared to the other methods, which makes it an alternative to the current methods of identifying these metal ions. On the other hand, this work has been applied in 2 ways: one with regard to the reagent in the field of antioxidants, and the other concerning the determination of cobalt in the filling of teeth. By conducting tests on the reagent as an anti-oxidant and anti-bacterial agent, it was

found that the reagent was highly effective as an anti-oxidant through the results that it showed in protecting the plasmid from oxidation by oxidizing agents. The organic compound also showed high efficacy as an anti-bacterial when its effect was compared with known antibiotics.

## Conflict of Interests

The authors declare that no competing interest exists.

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