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Antibacterial Activity of Cu (II) and Co (II) Complexes of 3, 4-Dihydroxybenzeneacrylic Acid Against the Pathogen, Nonpathogenic Bacteria and Sonochemical Synthesis of Nanoscale Mixed –Ligand EDA Coordination for Preparation of $CoCl_2.6H_2O$ Nanoparticle

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Abstract: Cu (II) and Co (II) metal complexes of 3, 4-dihydroxy benzene acrylic acid (EDA) as ligand were prepared in dry acetonitrile. These complexes were tested for their antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli* comparatively with that of free ligand. Differential response to phenolic compounds was observed among bacteria. Also these complexes were synthesized in Nano- scale and were characterized by SEM, XDR (X- Ray powder diffraction). Antibacterial activity of complexes and nano- material were studied and compared with each other.

Key words: 3, 4-dihydroxy benzene acrylic acid · Cu (II) and Co (II) complexes · Antibacterial activity · Nano- scale

INTRODUCTION

Acrylic acid or hydroxycinnamic acid compounds are widely distributed in the plants. They usually exist as esters of organic acid or glycosides or are bound to protein and other cell wall polymers. Only a small number of them exist as free acids in nature [1-3]. Much work has been realized by bioinorganic as well as medicinal chemists to launch the relationship between the metal ions and their complexes as antimicrobial agents [4, 5]. Phenolic compounds are secondary plant metabolites and naturally present in almost all plant materials, including food products of plant origin. These compounds are thought to be an integral part of both human and animal diets [6]. The chemical structure of phenolic acids shows that they are simple phenols. Hydroxycinnamic acid is the major subgroup of phenolic compounds [7, 8]. Hydroxycinnamates are phenylpropanoid metabolites and occur widely in plants [1] and plant products [9].

Hydroxycinnamates and their derivatives are bioactive plant food ingredients. The other natural ligand from plants such as alkaloids compound also can be used in synthesis of metal complex [10]. Nanophasic and nanostructured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications [11]. Nanoparticles are made of natural or artificial polymers ranging [12]. In particular, those conjugated with biological moieties have enormous potential in drug delivery and therapeutic applications. In fact, much progress has been achieved in the past ten years based on inorganic nanomaterials [13].

In this context we have undertaken the antimicrobial evaluation of Cu (II) and Co (II) complexes of 3, 4-dihydroxy benzene acrylic acid. For this purpose the *in vitro* susceptibility of tow gram positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*) and tow gram negative bacteria *(Pseudomonas aeruginosa, Escherichia coli*) to the synthesized compounds was investigated.

MATERIALS AND METHODS

Synthesis of the Metal Complexes; General Method: 3,4-dihydroxy benzene acrylic acid, cobalt chloride and copper chloride, were Merck chemicals and were used

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without further purification. Organic solvents were reagent grade. Electronic spectra were recorded by Camspec UV–Visible spectrophotometer model Perkin Elmer Lambda 25. The IR spectra were recorded using FT-IR Bruker Tensor 27 spectrometer. ^{1}H- NMR was recorded on a Bruker AVANCE DRX 500 spectrometer at 500 and 125MHz respectively. All the chemical shifts are quoted in ppm using the high-frequency positive convention; ^{1}H -NMR spectra were referenced to external $SiMe_4$. The percent composition of elements was obtained from the Microanalytical Laboratories, Department of Chemistry, University of tarbiyat-e-moallem, Tehran.

A solution of metal salt dissolved in acetonitrile added a dually to a stirred acetonitrile solution of the ligand (EDA), in the molar ratio 1:1 (metal: ligand). The reaction mixture was further stirred for 4-5h to ensure of the completing and precipitation of the formed complexes. Finally, the complexes dried in vacuum desiccators over anhydrous $CaCl_2$.

Microorganisms and Culture Media: The following microorganisms were used in this study to test antimicrobial activity of complex. *Escherichia coli*, *Staphylococcus aureus* were kindly provided by (Institute Pasteur in Tehran, Iran). The strains were maintained on PD_3 agar at 26°C. For long- term storage, glycerol stocks of microorganisms were prepared in the corresponding growth media with a final glycerol concentration of 12%. The bacterial glycerol stocks were quickly frozen in liquid nitrogen and stored at -80°C.

***In-vitro* Anti-Bacterial Activity:** All methodology and steps were followed according to diffusion disk method. An inoculum of 0.5 McFarland standard ($1.5*10^8$ cfu/ml) was applied on Mueller Hinton agar (a depth of 4 mm in a Petri dish of 100 mm diameter) [14]. Maximum 6 discs were applied on each plate and they were incubated at 37°C for 24 hours. Zone of inhibition was measured including the disc diameter (6mm).

Preparation of Nanoparticles: Co nanocrystallites were prepared by the reaction of $C_9H_8O_4$ with [Co ($C_9H_7O_4$)] Cl_2 in THF as solvent under ultrasound power. Then the suspension was irradiated for 1h with a high- density ultrasonic probe immersed directly into the solution under various conditions. A multi wave ultrasonic generator (sonicator – 3000: Italstructure MPD 3000). The samples were characterized with a scanning electron microscope (SEM) with gold coating.

RESULTS AND DISCUSSIONS

Structural Description of the Complexes: The reaction of Co(II) and Cu(II) salts with the ligand, EDA, results in the formation of [ML] for M=Co (II) and Cu(II). All complexes are quite stable and could be stored without any appreciable change. The EDA ligand and the $[Co(C_9H_6O_4)]Cl_2.2H_2O$ complex have 223-225°C and 195-198°C melting point respectively, but the $[Cu(C_9H_6O_4)]Cl_2.2H_2O$ complex have 169-172°C melting point. [Co ($C_9H_6O_4$)]$Cl_2.2H_2O$ complex is insoluble in common organic solvents, such as n-hexane, dichloromethane, but [Cu ($C_9H_6O_4$)] $Cl_2.2H_2O$ complex is insoluble in common organic solvents, such as dichloromethane. However, they are soluble in DMSO, ethanol and DMF. Their structures were characterized by elemental analysis, ^{1}H-NMR and IR. Their elemental analyses are in accord with their proposed formula. The spectral data of the complexes have good relationship with the literature data.

Analysis of [Co ($C_9H_6O_4$)] $Cl_2.2H_2O$ (EDACC): Dark Blue crystals; yield 86%. Elemental analyses, 1:1 metal to ligand stoicheiometry is assigned to all the chelates (Table 1).

^{1}H-NMR (ppm DMSO, 500MHz): δ 5, 97-7.21 [5H, 2q, aroma]; δ 11.91 [1H, s, acid) and δ 8.98-9,36[2H, s, alkene]. IR absorptions (cm^{-1} KBr): 1620 (C=C), 972 (=C-H), 1352 (C-O), 574 (Co-O) and 456 (Co-Cl). The electronic spectral data of the complex in acetonitrile are presented in Table 2. There are one peak in spectrum of ligand which can be assigned to $\pi \rightarrow \pi^*$ transition. The electronic complex shows a broad band at 680 nm attributable to the $^4T_{1g}$ (F) → $^4A_{2g}$ (F) and the other one at 640- 550 nm attributable to the $^4T_{1g}$ (F) → $^4T_{1g}$ (P) transition for Co (II) ion.

Raman Shift (cm^{-1}): 500 (Co-O), 325 (Co-Cl), 975(C-H), 1618(C=C), 1189(C-O) (Fig. 2. Left). Analysis of [Cu ($C_9H_6O_4$)] $Cl_2.2H_2O$ (EDACC): Orange crystals; yield 95%.

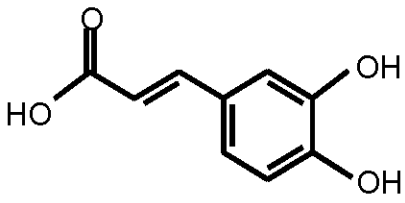

Fig. 1: Structure of the ligand, EDA

Table 1: Elemental analyses data on the caffeic acid and its Co (II) complex

	Elemental analyses %Found (cal.)		
Compound	C	H	Co
$C_9H_8O_4$	78.28	5.68	-
	(79.41)	(5.88)	-
CoL	32.06	1.68	16.25
	(31.40)	(1.74)	(17.15)

Table 2: Electronic spectra of caffeic acid and its Co (II) complex in nm

Compound	Interligand and charge transfer (CT)			d-d
Solvent	210	-	-	-
CoL	240	280-320	640-550	680

Table 3: Electronic spectra of caffeic acid and its Cu (II) complex in nm

Compound	Interligand and charge transfer (CT)		d-d
Solvent	210	-	-
CoL	240	280-330	470

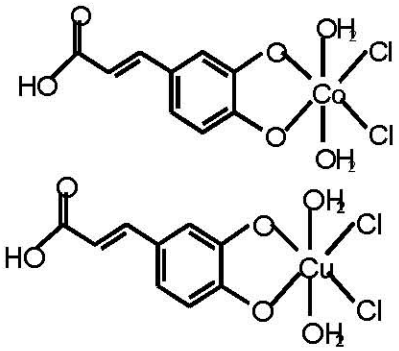

Fig. 2: Structure of Co (II) and Cu (II) complexes with ligand, EDA

Ir Absorptions (cm^{-1} KBr): 1609 (C=C), 897 (=C-H), 1281(C=O), 565(Cu-O), 445(Cu-Cl). The electronic spectral data of the complex in acetonitrile are presented in Table 3.

There are one peak in spectrum of ligand which can be assigned to $\pi \rightarrow \pi^*$ transition. The electronic complex shows a broad band at 470 nm attributable to the $^2Eg \rightarrow {}^2T2g$ transition for Cu (II) ion.

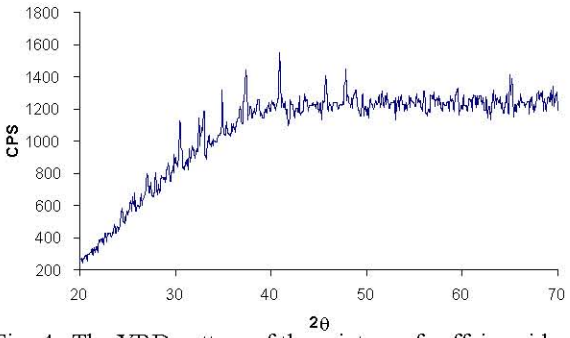

Fig. 4: The XRD pattern of the mixture of caffeic acid and $CoCl_2.6H_2O$

***In-vitro* Anti-Bacterial Activity:** The mean diameters of microbial growth inhibited by different complexes are shown in Table 4. All complexes had antimicrobial activity. Inhibition zones larger than 5mm indicated that antimicrobial activity. The data obtained by the disk diffusion method showed that all complexes have antibacterial activity. Among the bacteria, *Escherichia coli* was the most sensitive bacteria both ligand and complex $[Co(C_9H_7O_4)]Cl_2$- Normal- scale had antibacterial effect on this bacteria, whereas only ligand $C_9H_8O_4$ had antibacterial activity against *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. Ligand $C_9H_8O_4$ and complex $[Co\ (C_9H_7O_4)]\ Cl_2$ (Normal- scale) had not any effect against *Staphylococcus aureus*.

No antibacterial activity of Ligand was observed against *Staphylococcus aureus* whereas complex $[Cu\ (C_9H_7O_4)]\ Cl_2$ showed high activity against these bacteria (15mm). Complex $[Co\ (C_9H_7O_4)]\ Cl_2$ (Normal– scale), had more antibacterial activity against *Escherichia coli* (14mm). Complex $[Co(C_9H_7O_4)]Cl_2$ (Nano – scale), had less activity against *Staphylococcus aureus* (5mm) in comparison with complex $[Cu(C_9H_7O_4)]Cl_2$ that had more activity against this bacteria (15mm). From these results it may be concluded that there is not any accordance between normal – scale and nano- scale from the antibacterial activity aspect. The antimicrobial activity of complexes demonstrated in this study can be added to the already known beneficial biological properties of these compounds to the human health.

Table 4: Zone of growth inhibition of the test compounds against the bacteria

	Zone of growth inhibition (mm)			
Complexes	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Escherichia coli*
$C_9H_8O_4$	5	-	5	5
$[Cu(C_9H_7O_4)]Cl_2$	-	15	-	-
$[Co(C_9H_7O_4)]Cl_2$ Normal- scale	-	-	-	14
$[Co(C_9H_7O_4)]Cl_2$ Nano- scale	-	4	-	-

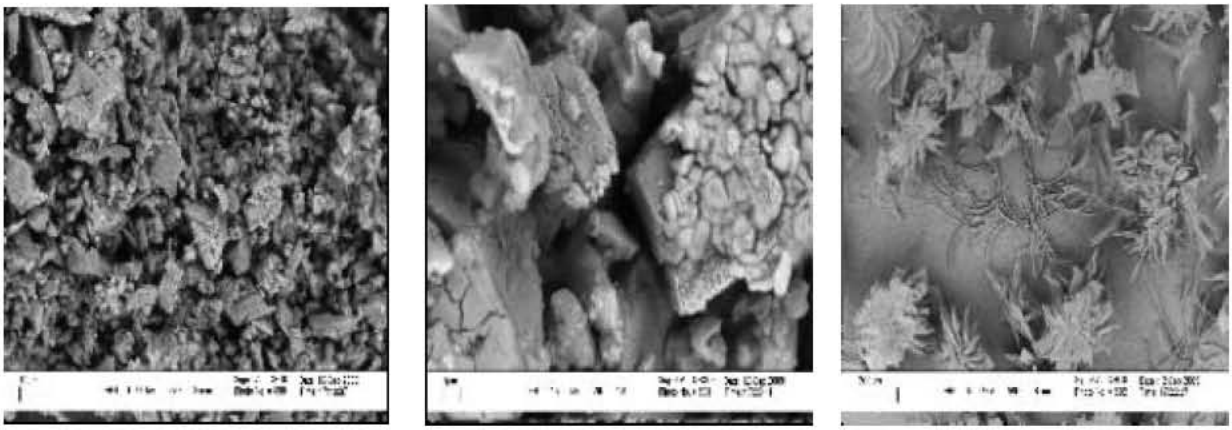

Fig. 5: SEM images of complex [Co ($C_9H_7O_4$)] Cl_2

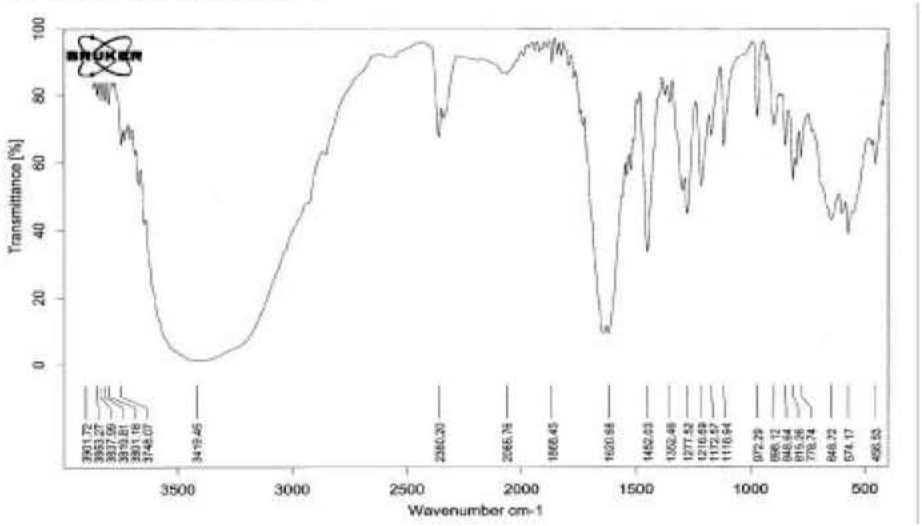

Fig. 6: IR Spectrum of complex [Co ($C_9H_7O_4$)] Cl_2

Nanoparticles Study: XRD pattern of mixture of caffeic acid and $CoCl_2.6H_2O$ prepared by the ultrasonic process is given in Fig. 4. The diffraction peaks accord with the amorphous crystal system.

The SEM micrographs of nanostructure are shown in Fig. 5. The nanoparticles show a low degree of crystalline with no defined peaks in the XRD pattern.

The IR spectrum of Co (II) nanostructure (Fig. 5) shows the absorption pesk at 574 - 648 are assigned to the ν (Co-O) modes, which confirms the formation of Co (II) nanostructure.

It has been reported that the negative charge on the cell surface of Gram-negative bacteria was higher than on Gram-positive bacteria. Due to a higher negative charge on cell surface, the interaction between Gram-negative bacteria and nanoparticles was definitely stronger than that of Gram-positive bacteria. Moreover, results showed that Gram-positive bacteria were more sensitive to Co (II) nanoparticles.

CONCLUSION

3, 4-dihydroxy benzene acrylic acid abbreviated as EDA and complex [Cu ($C_9H_7O_4$)] Cl_2, complex [Co ($C_9H_7O_4$)] Cl_2 in Nano and Normal – scale, were synthesized and characterized. All of these complexes had antibacterial effect. Gram-positive bacteria were more sensitive to Co (II) nanoparticle. Moreover, Gram-positive bacteria were more sensitive than Gram-negative bacteria.

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