# Synthesis, characterization and antibacterial activities of Mo(VI) and Cu(II) complexes derived from tridentate ONO Schiff base ligands

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## Abstract

Dioxomolybdenum(VI) and copper(II) complexes were synthesized using $H_2L^1$ and $H_2L^2$ tridentate Schiff base ligands. The ligands were derived from the condensation 2-hydroxy-4-methoxysalicylaldehyde or 2-hydroxy-4-methoxyacetophenone with 2-aminopropanol, respectively. Both complexes were characterized by physico-chemical and spectroscopic methods. Dimeric nature of the Mo(VI) complexes was revealed by IR spectroscopy. Furthermore, $MoO_2L^2$(VI) nanoparticles were synthesized by Ultrasonic irradiation.. Elemental analysis, scanning electron microscopy, and FT-IR spectroscopy were used to characterize the nanoparticles. The Schiff base ligands and their complexes have been studied for antibacterial activity against Gram positive and Gram negative bacteria. The results indicated that the Mo(VI) and Cu(II) complexes were effective against all of studied bacteria and its effectiveness was higher for, Escherichia coli.

**Keywords**:   Antibacterial activities; Molybdenum Schiff base complexes; nanoparticles; Ultrasonic irradiation.

## 1.Introduction

Schiff base compounds are one of the important groups of ligands in coordination chemistry with wide applications in various fields of science. The metal complexes of these ligands attract a great attention due to their simple preparation, vast applications, steric and electronic properties [1-3]. Furthermore, Schiff base ligands can be easily coordinated many various metals to stabilize them in different oxidation states [4-5]. Many Schiff bases and their complexes have received considerable interest due to their biological activity such as antibacterial, antifungal, antitumor and antimalarial [6-9]. It has been suggested that in such compounds the azomethine group is responsible for the biological activities [10]**.** In addition**,** Schiff base complexes have shown other applications such as

catalytic activity, photochromic properties, and transfer of oxygen [11-15]. In recent years, some components such as penicillins, nitrofuranes, anti-bacterial sulfa drugs, nitrosulfa, cephalosporins, tetracyclines, oxaolidinones are used as antimicrobial agents [16-17]. It is revealed that more than 70% of bacterial infections are resistant to at least one used antibiotics for eradication of the infection [18]. Despite much progress, there are still many problems in antimicrobial therapies which should have been solved for most of the available antimicrobial drugs [19]. It is necessary to search more effective antimicrobial agents and some Schiff bases because they have been known as promising antimicrobial agents [20].

Great opportunities and possibilities have lately been offered by nanotechnology in the various fields of

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science and technology. Nowadays, many researchers have increasingly paid attention to pharmaceutical nanotechnology due to its numerous advantages [21]. Generally speaking, nanoparticles have various properties compared to the bulk materials. Indeed, the ratio of surface to volume of the nanoparticles is significantly increases with the decreasing of the particle size [22]. It means that fraction of the molecule surface in the nanometer dimensions considerably increase and it turn improves some properties of the particles such as dissolution rate, catalytic activity, mass transfer [23]. During these years, more researches have been synthesized metal nanoparticles, metal oxides, sulfides and ceramic materials [24-28]. Ultrasonic irradiation is one of most promising techniques for achieving nanoparticles [29-30]. However, researchers do not more attentions to nanostructures of supramolecular compounds.

In the present work, Mo(VI) and Cu(II) complexes, and nanostructured Mo(VI) complex have been synthesized under ultrasonic irradiation. The Schiff base ligands and their complexes were evaluated for their antibacterial activity against Gram positive and Gram negative bacteria.

## 2. Experimental

### 2.1 Materials and measurements

All the chemicals and solvents with analytical reagent grade, were purchased from Aldrich, Merck or Fluka and used without any further purification. $MoO_2(acac)_2$ and the ligands were prepared as described in the literature [31, 32]. NMR and FT-IR (KBr pellets, 450-4400 cm$^{-1}$) spectra were obtained using a Bruker 400MHz DRX spectrometer and a Bruker FT-IR instrument, respectively. Moreover, Microanalyses of the complexes were carried out by a LECO 600 CHN elemental analyzer. Thermogravimetric (TG) analysis of the synthesized complexes was performed on a Perkin Elmer analyzer. The TG analyzer was regulated under nitrogen atmosphere at heating rate of 10 ˚C /min at a temperature ranging of 50-500 ˚C. The molar

conductivities were determined at 25°C with a Jenway 400 for Mo(VI) and Cu(II) complexes in 10$^{-3}$ M MDF solutions. Ultrasonic generators were carried out on a SONICA-2200 Ep, input 50- 60 Hz/305w.

### 2.2 Preparation of complexes

### 2.2.1 Preparation of molybdenum(VI) complex, $MoO_2L^1(VI)$, 1

The $MoO_2L^1(VI)$ complex was prepared as follows: Firstly, $MoO_2(acac)_2$ ( 0.322 g, 1mmol) was dissolved in methanol (10 ml) and then was added to a solution of Schiff base ligand, $H_2L^1$ (0.215g, 1mmol) dissolved in methanol (10 ml). The obtained mixture was refluxed for 2 h and finally, the yellow precipitate was isolated. The synthesized complex was recrystallized from methanol. (Yield: 95%, 0.328 g, (Scheme 1)). Selected IR data (υ/cm$^{-1}$ ): 1605 (C=N), 1546 (C=C), 1233 (C-O), 912 and 934 (Mo=O), 825 (Mo=O---Mo). $^1$HNMR(400 MHz, DMSO, 22ºC), δ(ppm): 7.44 (1H, d, J=8.8, Ar), 6.54 (1H, dd, J$_1$=8.6, J$_2$=2.8, Ar), 6.43 (1H, d, J=2.4, Ar), 4.45 (1H, m, CH$_2$**CH**(OH)CH$_3$), 4.11 (1H, dd, J$_1$=14, J$_2$=4.0 **CH**$_a$H$_b$CH(OH)CH$_3$), 3.52 (1H, ddd, J$_1$=13, J$_2$=10, J$_3$=2.0, CH$_a$**H**$_b$(OH)CH$_3$), 8.57 (3H, S, **CH**=N), 1.25 (3H, d, J=6.0, CH$_2$CH(OH)**CH**$_3$). $^{13}$C NMR (400 MHz, DMSO, 22ºC), δ(ppm): 165.23, 163.92, 162.97, 135.43, 115.25, 107.98, 103.38, 78.01, 67.12, 55.98, 20.30. Anal.Calc. for C$_{11}$H$_{13}$MoNO$_5$ (MW=345 g/mol): C, 39.46; H, 3.88; N, 4.18; found: C, 39.33; H, 3.84; N, 4.25
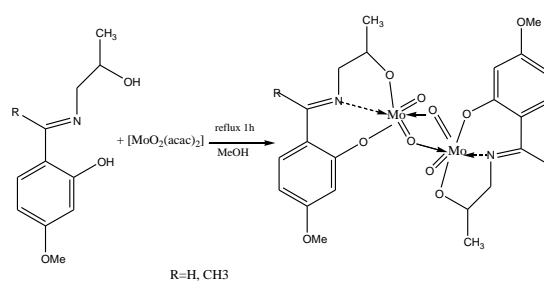


**Scheme 1.** Synthesis of $MoO_2L$ (VI) complex.

### 2.2.2 Preparation of molybdenum(VI) complex, $MoO_2L^2(VI)$, 2

The $MoO_2L^2(VI)$ complex was prepared as follows: $MoO_2(acac)_2$ ( 0.322 g, 1mmol) was dissolved in

methanol (10 ml) and was added to a solution of Schiff base ligand, $H_2L^2$, (0.223g, 1mmol) dissolved in methanol (10 ml). The mixture was refluxed for 2 h and at the end, the yellow precipitate were isolated. The synthesized complex was recrystallized from methanol. (Yield: 81%, 0.848 g, (Scheme 1)). Selected IR data ( $\upsilon/cm^{-1}$): 1591 (C=N), 1545 (C=C), 1249 (C-O), 928 (Mo=O), 817 (Mo=O--- Mo). $^1$HNMR(400 MHz, DMSO, 22ºC), δ(ppm): 7.71 (1H, d, J=8.8, Ar), 6.50 (1H, dd, $J_1$=8.8, $J_2$=2.8, Ar), 6.40 (1H, d, J=2.8, Ar), 4.49 ( 1H, m, $CH_2$**CH**(OH)$CH_3$), 4.09 (1H, dd, $J_1$=13.8, $J_2$=4.0, $CH_a$**Hb** CH(OH)$CH_3$), 3.35-3.309 (1H, dd, $CH_a$**Hb** (OH)$CH_3$), 2.48 (3H, S, **CH$_3$**(C=N), 1.26 (3H, d, J= 6.4, $CH_2$CH(OH)**CH$_3$**). $^{13}$C NMR (400 MHz, DMSO, 22ºC), δ(ppm): 169.26, 163.49, 131.85, 118.176, 107.59, 103.2, 78.19, 61.95, 55.84, 20.6. Anal.Calc. for $C_{12} H_{15}MoNO_5$ (MW=349 g/mol): C, 41.26; H, 4.29; N, 4.01; found: C, 41.32; H, 4.33; N, 4.02.

## 2.2.3 Preparation of copper(II) complex, [Cu(HL$^1$)Cl]$_2$.H$_2$O, 3

The $CuL^1$(II) complex was prepared as follows: $CuCl_2.2H_2O$ (0.256 g, 1.5 mmol) was dissolved in methanol (10 ml) and was added to a solution of Schiff base ligand, $H_2L^1$ (0.322 g, 1mmol) dissolved in methanol (10 ml). The mixture was refluxed for 2h, after some days, green microcrystals was formed which was removed by filtration, washed with cooled methanol, recrystallized from methanol and vacuum dried. (Yield: 66%, 0.41 g, (Scheme 2)). Selected IR data ($\upsilon/cm^{-1}$): 3429 (O-H), 1595 (C=N), 1532 (C=C), 1238 (C-O). Anal. Calc. for $C_{22}Cl_2Cu_2H_{28}N_2O_7$ (MW=625 g/mol): C, 41.77; H, 4.43; N, 4.43; found: C, 42.45; H, 4.80; N, 4.40.
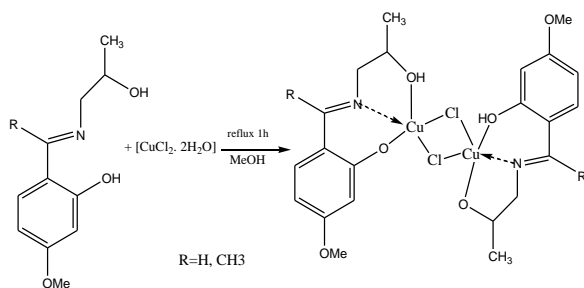


**Scheme 2**. Synthesis of CuL (II) complex

## 2.2.4 Preparation of copper(II) complex, [Cu(HL$^2$)Cl]$_2$.H$_2$O

The Cu(II) complex was prepared as follows: $CuCl_2.2H_2O$ (0.256 g, 1.5 mmol) was dissolved in methanol (10 ml) and then was added to a solution of Schiff base ligand, $H_2L$ (0.223g, 1mmol) dissolved in methanol (10 ml). The mixture was refluxed for 2h resulting in green microcrystals was formed after some days, which was removed by filtration, washed with cooled methanol, recrystallized from methanol and vacuum dried. (Yield: 43%, 0.57 g, (Scheme 2)). Selected IR data ($\upsilon/cm^{-1}$): 3429 (O-H), 1595 (C=N), 1532 (C=C),1238 (C-O). Anal. Calc. for $C_{24}Cl_2Cu_2H_{32}N_2O_7$ (MW=660 g/mol): C, 43.63; H, 4.84; N, 4.24; found: C, 43.85; H, 5.3; N, 4.11.

## 2.2.5 Preparation of molybdenum complex at nano-size in a sonochemical process

Mo (VI) complex was prepared as described in the literature [29]. Briefly, to prepare nanostructured dioxomolybdenum Schiff base complex a solution of $H_2L$ ligand (0.223 g, 1mmol) in 10 ml of chloroform was added dropwise into a solution of $MoO_2(acac)_2$ ( 0.322 g, 1mmol) in 30 ml methanol during 40 min under ultrasonic irradiation. The solution was kept in the ultrasonic bath during of 40 min. The obtained yellow precipitates were filtered, and then washed with cooled methanol, and finally vacuum dried (Yield: 93%, 0.324 g).

## 2.3 Antibacterial activity assay

The in vitro biocidal screening, antibacterial activities of the Schiff base ligands, $H_2L^1$, $H_2L^2$, and Mo (VI) and Cu(II) complexes were assayed using Kirby–Bauer disc diffusion method where a filter disc was impregnated with the compounds and placed on the surface of inoculated agar plates [33]. The synthesized compounds were dissolved into DMSO to achieve 20 mg mL$^{-1}$ solution then filter sterilized using a 0.22 μm Ministart (Sartorius).

The antibacterial activity of the compounds was investigated against four bacterial species. Test

organisms included *Escherichia coli* PTCC 1330, *Pseudomonas aeruginosa* PTCC 1074, *Staphylococcus aureus* ATCC 35923 and *Bacillus subtilis* PTCC 1023 [34]. Late exponential phase of the bacteria were prepared by inoculating 1% (v/v) of the cultures into the fresh Muller-Hinton broth (Merck) and incubating on an orbital shaker at 37 °C and 100 rpm overnight. Before using the cultures, they were standardised with a final cell density of approximately $10^8$ cfu mL$^{-1}$. Muller-Hinton agar (Merck) were prepared and inoculated from the standardized cultures of the test organisms then spread as uniformly as possible throughout the entire media. Sterile paper discs (6 mm diameter, Padtan, Iran) were impregnated with 20 µL of the compound solution. The impregnated disc was introduced on the upper layer of the seeded agar plate and incubated at 37 °C for 24 hours. The antibacterial activities of the compounds were compared with known antibiotic gentamicin (10 µg/disc) and chloramphenicol (30 µg/disc) as positive control and DMSO (20 µL/disc) as negative control. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (mm) on the surface of plates and the results were reported as Mean ± SD after three repeats.

### 2.3.1. Statistical analyse*s*

Statistical analyses were performed via PASW Statistics program package, version 18 (SPSS Inc., Chicago, IL, USA). Comparison of obtained data for compounds was performed using One-Way ANOVA followed by Tukey posthoc test. The significance level was set at p>0.05 and p>0.001.

### 3. Results and Discussion

### 3.1 Characterization of Cu(II) and Mo(VI) Schiff base complex

All the complexes in this work have been prepared with the addition of a methanolic solution of Schiff base ligand, $H_2L^1$ and $H_2L^2$ to a methanolic solution of the metal ion at a ratio 1:1 for Cu(II) and Mo(VI). The Schiff base ligands acts as tridentate ONO donor ligand towards $MoO_2^{2+}$ and $Cu^{2+}$ centers. These complexes are

stable and soluble in Methanol, DMF and DMSO solvents however they are insoluble in chloroform and dichloromethane solvents. The experimental and calculated elemental analysis data are in good agreement each other. Furthermore, the molar conductivity of $10^{-3}$ M solutions of the complexes in DMF indicated that all the metal complexes had conductivity values ranging from 5.2 to 30.9 ohm$^{-1}$ cm$^2$ mol$^{-1}$, which suggests these complexes are non-electrolyte [35]. The thermal studies of the synthesized Mo(VI) and Cu(II) complexes gave information about thermal stability these coordination compounds. In fact, the thermal traces gave very beneficial information about the number and nature of water molecules in complexes [36-37]. In the case of the $MoO_2L$ (VI) complexes (Figs. 1 and 2), no mass loss was observed in the region below 150°C which indicated the absence of lattice water molecules. TG curves of Cu(II) complexes (Figs. 3 and 4) show the weight loss of one water molecule in the range of 80-140 °C which suggest the presence of lattice water molecules in these complexes. According to TG curves of these complexes, there is a mass loss in the temperature ranging from 170 to 360 °C due to the decomposition of the ligands.
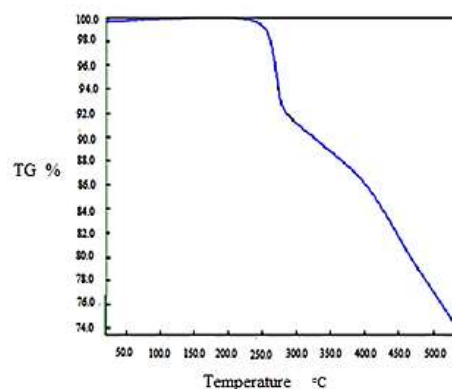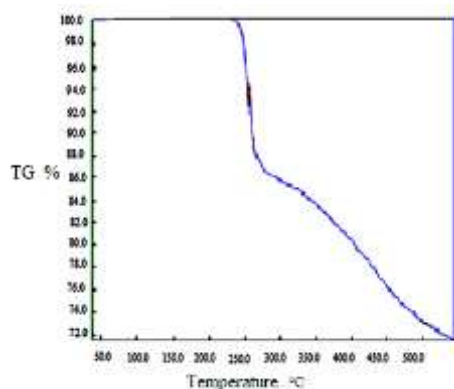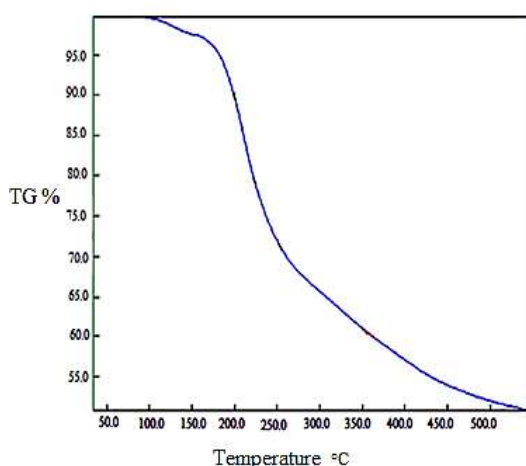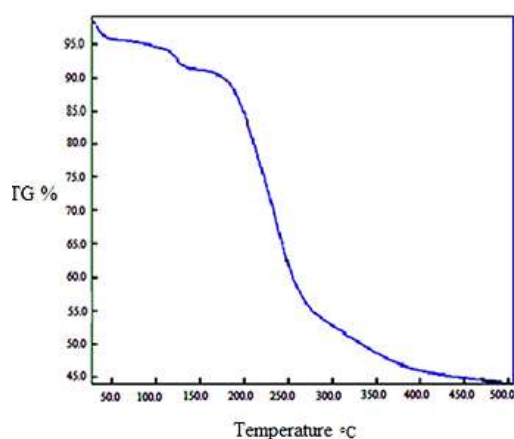


**Fig. 1.** TG plot of $MoO_2L^1$(VI) complex

**Fig. 2**. TG plot of $MoO_2L^2$(VI) comple



**Fig. 3**. TG plot of $[Cu(HL^1)Cl]_2 \cdot H_2O$



**Fig. 4**. TG plot of $[Cu(HL^2)Cl]_2 \cdot H_2O$

The Mo(VI) and Cu(II) complexes were characterized by FT-IR (as listed in Table 1). The infrared (IR) spectra of $MoO_2$(VI) and Cu(II) complexes, were compared with those of the their ligands which helped us to understand coordination mode of ligand to the metal. The Schiff base is able to form coordinate bonds with metal ions through both phenolic group and

azomethine group. The strong peaks of the free ligands at 1645 and 1602 $cm^{-1}$ can be assigned to the $\upsilon$ (C=N) (azomethine) where the peaks were shifted to lower frequencies and were appeared at new wavenumbers of 1635, 1591 and 1595 $cm^{-1}$ in the spectra of the complexes (indicating the coordination of azomethine nitrogen to the metal) [38-40]. The stretching vibrations of $OCH_3$ group appeared at 2923- 2960 $cm^{-1}$ region. IR spectra of the Cu(II) complex showed broad band around 3456 $cm^{-1}$ which suggests the presence of lattice water molecule [41]. The IR spectra of the Mo complexes exhibited strong M=O bands in the region 912 to 934 $cm^{-1}$ due to O=Mo=O stretch [42]. The presence of sharp bands at 825 and 817 $cm^{-1}$ indicated the formation dimeric dioxomolibdenum complexes **1** and **2**, respectively [37, 38]. Dimeric dioxomolibdenum (VI) complex of tridentate ligands have sharp bands in the region of 800-850 $cm^{-1}$ as were reported at previous researches [37, 43]. The IR spectra and elemental analysis of the $MoO_2L$ complexes as nanostructure and bulk show much similarity which suggested identical structures for the molybdenum complexes (Fig. 5).

**Table 1**. IR spectral data of the ligands $H_2L^1$ and, Cu(II) and Mo(VI) complexes

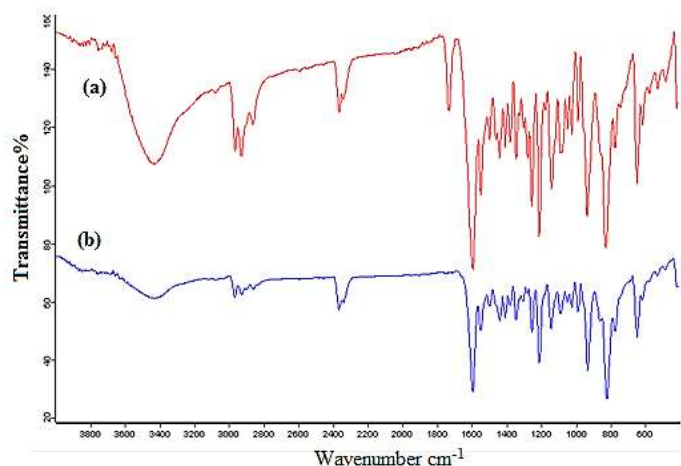| Compound | Selected IR bands ($cm^{-1}$) | | | | |
|---|---|---|---|---|---|
| | $\upsilon$ (C=N) | $\upsilon$ (C-O) | $\upsilon$ ($OCH_3$) | $\upsilon$ (Mo=O) | $\upsilon$ (Mo=O---Mo) |
| $H_2L^1$ | 1643 | 1219 | 2855-2964 | - | - |
| $H_2L^2$ | 1645 | 1219 | 2853-2962 | - | - |
| $MoO_2L^1$ | 1635 | 1233 | 2934-2970 | 912, 934 | 825 |
| $MoO_2L^2$ | 1591 | 1249 | 2859-2964 | 928 | 817 |
| $CuL^1$ | 1595 | 1238 | 2929 | - | - |
| $CuL^2$ | 1595 | 1238 | 2929 | - | - |
| Nano-$MoO_2L$ | 1591 | 1250 | 2859-2962 | 928 | 817 |

**Fig. 5**. IR spectra of $MoO_2L^2$-nano (a) and $MoO_2L^2$-bulk (b).

The electronic spectra of the complexes showed two absorption bands in the region of about 280- 364 nm. These bands were assigned to the π-π* and n-π* transitions. The observed first absorption band at 280, 281,282 and 286 nm were related to $CuL^1$(II), $MoO_2L^1$(VI), $CuL^2$(II) and $MoO_2L^1$(VI) complexes, respectively which were attributed to π-π* transition. The second band appeared at 364, 346, 333 and 361 nm correspond to $CuL^1$(II),     $MoO_2L^1$(VI), $CuL^2$(II), $MoO_2L^2$(VI) and complexes, respectively and were due to the n-π* transitions [29].

The $^1$H NMR spectra of the free Schiff base ligands, $H_2L^1$, $H_2L^2$ shows a singlet signal at 13.72 and 12.8 ppm, respectively which can be attributed to phenolic -OH proton. The peak corresponding to phenolic -OH disappeared in the spectra of the complexes, which supported deprotonation of the ligands to coordinate to the molybdenum center. The protons due to –$OCH_3$ have appeared as singlets at 3.78 - 3.77 ppm with an area integral of three  in which indicated the presence of three methoxy hydrogen atoms in the $MoO_2L^1$(VI) and   $MoO_2L^2$(VI) complexes, respectively. The $MoO_2L$(VI) complexes showed a multiple in the region of   6.39 - 7.71 ppm corresponding to the aromatic protons.   The $^1$H NMR spectrum of $MoO_2L^1$(VI) complex showed a singlet  peak at 8.57 ppm which could be attributed to the  iminic poroton (CH=N). In the $^1$H NMR spectrum of $MoO_2L^2$(IV) complex, the

singlet peak observed at 2.48 ppm was assigned to –($CH_3$ )C=N protons with an area integral of three. In the $^{13}$C NMR spectra of Mo(IV) complexes, the peaks corresponding to azometine carbon have shifted downfield and can confirm the coordination of azomethine nitrogen to metal ions. The signals of methoxy C atom ( –$OCH_3$) were appeared at  55.98 and 55.84 ppm in the both of the Mo (VI) complexes.

Scanning electron microscopy (SEM) measurement was carried out to obtain information of morphology, structure and size of as-synthesized nanostructures. Fig. 6A shows SEM images of nanostructures synthesized by the sonochemical method. SEM images showed that nanoroads were well distributed with the minimum and maximum size ranging from 25 to 74 nm and 68 nm to 1.301  μm  for  $MoO_2L$-nano  and  $MoO_2L$-bulk, respectively (Fig. 6A and B). It is confirmed that using of ultrasonic irradiation is perfect method to produce nanostructures with significant decrease in its size.
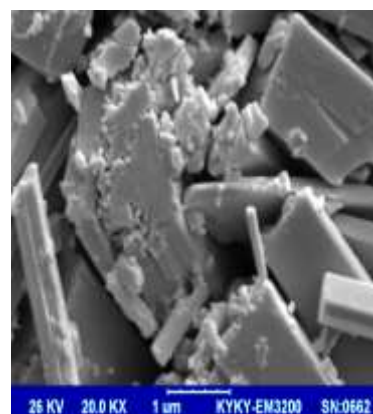
**(a)**
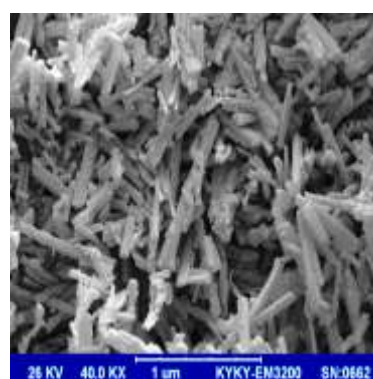


**(b)**



**Fig. 6**. The SEM images of $MoO_2L^2$(VI)-nano (A) and $MoO_2L^2$(VI)-bulk (B).

### 3.2 Antibacterial activity

The in vitro antibacterial activity of the ligands, $H_2L^1$ and $H_2L^2$,  and their complexes was evaluated against Gram positive *S. aureus* and *B. subtilis*, and Gram negative *E. coli* and *P. aeruginosa*. The finding towards inhibition of microorganisms was correlated with the standard antibiotics gentamicin and chloramphenicol (Table 2). The results indicated that, ligand $H_2L^2$ did not show any antibacterial activity, while its complexes had a good potential of antibacterial activity (Fig. 7and 8); besides, the ligand $H_2L^1$ was physiologically active and chelation made its activity be enhanced. The results revealed that the complexes have good growth inhibitory effect against some microorganisms. The values obtained for Cu(II) complexes represents good activity against all test bacteria, whereas the growth inhibitory of all complexes were shown low activity against *P. aeruginosa* (Table 2). As seen in Table 3, the antibacterial activity of the complexes on Gram-negative bacteria was less than its activity on Gram-positive ones. Increasing in antibacterial activity of Mo(VI) and Cu(II) complexes can be explained based on Tweedy's chelation theory [44- 46]. This probably can be attributed to the greater lipophilic nature of the complexes.
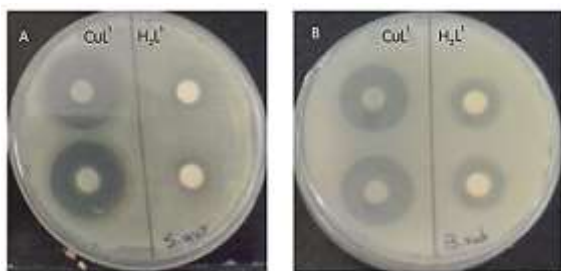


**Fig. 7**. Antibacterial activities of CuL[1] (II) comlex and $H_2L^1$ against *S. aureus* (A) and *B. subtilis* (B) using Kirby-Bauer disc diffusion method
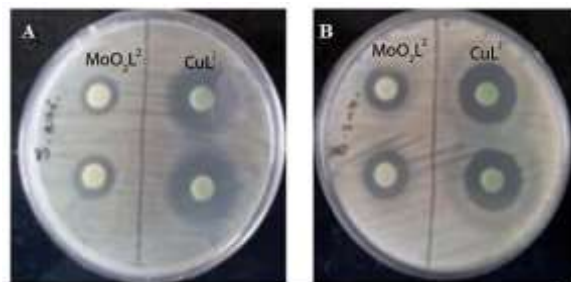


**Fig. 8.** Antibacterial activities of the $MoO_2L^2$(VI) and CuL[2](II) complexes against (A) *S. aureus* and (B) *B. subtilis* using Kirby-Bauer disk diffusion method.

| Compound | Zone of growth inhibition (mm) | | | |
|---|---|---|---|---|
| | *E. coli* | *P. aeruginosa* | *S. aureus* | *B. subtilis* |
| $MoO_2L^1$ | 30.0±1.4 [a] | 11.5±0.7 [a] | 11.5±0.7 [a] | 12.5±0.7 [a] |
| $MoO_2L^2$ | 12.5±0.7 [a] | 11.5±0.7 [a] | 12.5±0.7 [a] | 14.5±0.7 [a] |
| $CuL^1$ | 14.5±0.7 [b] | 12.5±0.7 [a] | 24.0±1.4 [b] | 20.5±0.7 [b] |
| $CuL^2$ | 16.5±0.7 [b] | 13.5±0.7 [b] | 22.0±1.4 [b] | 18.5±0.7 [b] |
| $H_2L^1$ | 20.5±0.7 [c] | 8.0±1.4 [b] | 9.5±0.7 [c] | 12.5±0.7 [a] |
| $H_2L^2$ | NE | NE | NE | NE |
| P-value ANOVA[2] | <0.001 | <0.001 | <0.001 | <0.001 |
| Gentamicin (10 µg/disc) | 19.6±1.1 | 15.6±0.5 | 20.3±1.5 | 26.0±1.7 |
| Chloramphenicol (30 µg/disc) | 20.7±1.5 | NE | 21.7±0.6 | 22.3±1.2 |

**Table 2**. Antibacterial activity of the Schiff base ligand and Cu(II) and Mo(VI) complexes using Kirby–Bauer disc diffusion method.
[1] *According to analysis of variances (p≤0.05) the difference between quantities with similar superscripts (a, b) and c) is not significant for data of each row.*
[2] *P-value ANOVA: Comparison between MoL, CuL and L*
[3] *No Effect*

### 4. Conclusions

Two complexes of Mo(VI) and Cu(II) containing tridentate ONO Schiff base ligands, $H_2L^1$ and $H_2L^2$ , have been synthesized and characterized using physicochemical techniques. The absorption peaks of IR spectroscopy shows dimeric nature of the $MoO_2L$ complexes. Thermal studies help us to understand the thermal stability and number and nature of water molecules in complexes. TG curve of Cu(II) complexes revealed the position of water molecule in outer sphere of coordination. The synthesized complexes indicate

good antibacterial activity against most test bacteria so in future we can develop them for using as effective antibacterial agents.

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**Highlights**

- Molybdenum Schiff base complexes
- Ultrasonic irradiation
- Antibacterial activity