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# The Antibacterial activity of new ethyl 2-oxo-2H-chromene-3-carboxylate complexes against bacteria isolate from wound infection in Baghdad city .

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**Abstract**
The related researches are based on preparation and mixed of some ligand complexes of the transitional metal ions [Cu(II), La (III)] with ethyl coumarin (ethyl 2-oxo-2H-chromene-3-carboxylate) , finally on the studies of antimicrobial (antibacterial) activities of the same. For their synthesis the prepared solutions of metal chlorides are allowed to react with an ethanolic solution of ethyl 2-oxo-2H-chromene-3-carboxylate in different ratio. Disc diffusion methods were employed for antimicrobial assays against four human pathogenic bacteria isolation from wound infection The bacterial properties of the metal complexes reveal that the Lanthanum complex is more effective against almost all antibacterial tested. Furthermore The *staphylococcus aureus* bacteria was more types of bacteria sensitive to the effect of the complex studied .
**Key words:** Antimicrobial activities, metal complexes**,** human pathogenic bacteria, wound infection

## 1. Introduction

Intact skin is the perfect defense to bacterial invasion, but damage to the skin allows bacteria, fungi and yeasts to enter. More than 200 different species of bacteria normally live on the skin **[ 1 ]** and an open wound provides a moist, warm and nutritious environment perfect for microbial colonisation and proliferation. Bacteria colonise all chronic wounds and low levels of bacteria can benefit the wound by increasing the amount of neutrophils, monocytes and macrophages in the wound, thus improving levels of prostaglandin E2 and the formation of collagen **[ 2 ]** . When one or more microorganisms multiply in the wound, local and systemic responses occur in the host, which can lead to infection and a subsequent delay in healing **[ 3,4 ]** .

Wound infections have been regarded as the most common nosocomial infections and are associated with increased morbidity and mortality **[5-7]**. Infection in a wound delays healing, causes wound breakdown, prolonged hospital stay, increased trauma care and treatment costs **[4,8]** . Bacteriological studies have also shown that wound infections is universal and that the types of bacteria vary with geographical locations, bacteria resident on the skin, clothing at the site of wound, time between wound and examination **[7,9]** . The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and due to an increasing incidence of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and polymicrobic flora **[ 7 ]** .

In developing countries, wound infections are recognized as a prominent route of bacterial infections. Many bacterial agents are known to cause wound infections **[ 10,11 ]** . Isolates that have been incriminated in cases of wound infections include: *Staphylococcus aureus*, *Proteus mirabilis* , *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Streptococcus faecalis*. *Candida albicans* and *C. tropicalis* have also been implicated as etiological agents **[ 12-15 ]** .

Synthetic chemical compounds constitute important sources of various bioactive compounds such as antibacterial, antifungal and anticancer compounds **[16-18]**. The synthesized chemical compounds, which are used for the treatment of infectious diseases are known as chemotherapeutic agents. Every year thousands of compounds are synthesized with an aim to find a potential chemotherapeutic agents to combat pathogenic microorganisms. But a very few compounds withstand as therapeutic agent by various methodological tests. Antimicrobial screening is one of these tests required to perform for primary selection of compounds as the therapeutic agents. Metal chelation or complexation is involved in many important biological process where the co-ordination can occur between a variety of metal ions and a wide range of ligand **[19]**. Many types of ligand are known and the properties of their derived metal chelate have been investigated **[20]**. Prior to 1980, search for anticancer drugs was focused primarily on organic compounds **[21]** . However, with the discovery of cis-diammine dichloro platinum (II) which shows excellent antitumor activity, keen interest arose in exploring other inorganic compounds. Copper, Silver and gold complexes are among the most promising inorganic compounds known to possess anticancer activity. Copper is found in human cells and is primarily associated with copper – dependent enzymes that are required for normal metabolic process. The complexation of Co, Fe, Mg, Zn and Cu with nitrogen containing chain in the enzymes are very diverse **[22]** . The antimalarial activities of a series of 2-

acetyl pyridine and their Cu, Ni, Fe, and Mn complexes have been tested for their antimalarial and antiteukemic properties. These compounds have been found to possess significant antimalarial activities **[23]** .

A review article dealing with the varied physiological activities of coumarin derivatives has been published, describing their anticoagulant, antibacterial, antihelminitic, hypothermal properties and vasodilatatory action. During the last twenty years, the study of the biological activities of coumarin derivatives has been the aim of many researchers. Also, the structure activity relationships of coumarins have revealed that the presence of substituted thiocarbonylmercaptoacetylamino derivatives is an essential feature of their pharmacological action. Based on these findings, we describe the synthesis of some compounds featuring different heterocyclic rings fused onto the coumarin moiety with the aim of obtaining more potent pharmacologically active compounds **[ 24 ]** .

Coumarin (2H-1-benzopyran-2-one), a naturally occurring plant constituent, has been used in the treatment of cancer and oedemas, and many of its derivatives have also shown biological activity. Biological effects observed include antibacterial, anti-thrombotic and vasodilatory, anti-mutagenic and anti-tumourigenic effects as well as acting as lipoxygenase and cyclooxygenase inhibitors . A number of recent studies have highlighted the antimicrobial activity of naturally derived and synthetic coumarins. Lately, a number of metal complexes of coumarins have been synthesised and their biological activity determined. Kostova et al. have shown the cytotoxicpotential of coumarins complexed with cerium, lanthanum, zirconium and neodymium. We have previously been concerned with two main areas of coumarin chemistry, namely the chemotherapeutic and antimicrobial activity of functionalised coumarins. In the latter work a series of copper(II) and silver(I) complexes of hydroxynitrocoumarins were prepared and their antimicrobial activity assessed against a series of Gram-positive and Gram-negative bacterial strains and also against a clinical isolate of C. albicans. While none of the coumarin-based ligands or the simple copper(II) perchlorate salt showed any significant antimicrobial activity, AgNO3 and its coumarin complexes effectively inhibited the growth of the clinically important methicillin-resistant Staphylococcus aureus (MRSA) bacterium. These complexes also demonstrated good activity, comparable to that of the commercial fungicides clortrimazole and ketoconazole, against the fungal pathogen C. albicans. Both of these human pathogenic organisms are of increasing importance with the development of resistance to current drug therapies **[ 25 ]** .

## 2. Experimental Work

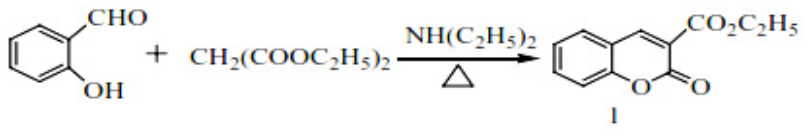
### 2.1 Test microorganisms

The bacteria (test organisms) were collected from the AL- Kadhimiya Teaching Hospital in Baghdad. All steps of the work were carried out at the microbiology laboratory, Department of biology , University of Al-Mustansiriyah. Four isolates of *staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative), *pseudomonas aeruginosa* (Gram negative) and *Proteus mirabilis* (Gram negative) were selected on the basis of higher frequency in wound infections , the organisms were identified by standard microbiological techniques including colonial characteristics, morphological characteristics and biochemical characteristics **[26] .**

### 2.2 Chemical Synthesis of ligands complex (complex 1, 2 and 3 )

#### 2.2.1 complex 1 : ethyl 2-oxo-2H-chromene-3-carboxylate (L)

Salicylaldehyde (1.22 g, 0.01 mol) and diethylmalonate (1.6 g, 0.01 mol) were dissolved in ethanol to give a clear solution. Piperidine (2 ml) was added and the mixture was refluxed for 5 h. The content was concentrated to a small volume. The product was poured onto crushed ice, filtered out and crystallized from ethanol to give colourless crystals

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#### 2.2.2 complex 2,3 : dichloro bis ( di ethyl 2-oxo-2H-chromene-3-carboxylate) copper (II) ( 2) , dichloro bis ( di ethyl 2-oxo-2H-chromene-3-carboxylate) Lanthanum (III) chloride ( 3 ) .

Added (0.14 grams and 0.371 grams, 1 mmole) of chloride each of copper and Lanthanum respectively and dissolved the lowest amount of ethanol absolute to ( 0.436 g , 2 mmoles ) of the compound 1 dissolved in 15 mL of solvent was then escalate the combination of each complex for three hours later filtered and washed with distilled water first and then hot ethanol and dried and then calculates the weight .

2.3 Antibacterial activity

The complexes were screened for antibacterial activity against *staphylococcus aureus* , *Escherichia coli*, *pseudomonas aeruginosa and Proteus mirabilis* . The activities were carried out with the help of disc diffusion technique. Each disc contained 200 µg / ml of compound and it was placed on bacteria inoculated plates. The growth inhibition results were compared with standard antibiotic Cefoxitin 30µg/disc and dimethylsulfoxide (DMSO), which were used as control .

Sterile filter paper discs were taken and the test material of known concentration was applied on the discs with the help of a micropipette. The solvents from the discs were evaporated by hot air blower. In the similar way control discs (containing only the solvents) were also prepared. The Muller-Hinton Agar plates were seeded with fresh culture with the help of a micropipette and spread the microorganisms with the help of a sterile spreader in an aseptic condition. The prepared discs of samples were placed gently on the freshly seeded Muller-Hinton Agar plates with a sterile forceps. Standard discs and control discs were also placed on the test plates to compare their effect with tested samples. Then the plates were kept in a refrigerator at 4 $C^{o}$ for 24 hours in order that the materials had sufficient time to diffuse to a considerable area of the plates. After this, the plates were incubated at 37 $C^{o}$ for 24 hours. After incubation, the diameter of the zone of inhibition were observed and measured in mm by a transparent scale.

## 3. Results and Discussion

Antibacterial activities (in vitro study) of these complexes were tested in the present studies and results are presented in Tables 1-3. The antimicrobial activity of the complex 1, 2 and 3 were determined at the concentration of 200 ∝g/disc against a series of Gram positive and Gram negative pathogenic organisms isolated from wound infection . From the zone of inhibition, it has observed that the complex 3 was more active than the others. The complex 1,2 also has showed substantial antimicrobial activity. It may conclude that most of the complexes have antibacterial effect except complex no. 1, which has less antibacterial effect. By the results that appeared to have conclude that the addition of chloride each of copper and lanthanum to the parent complex led to increased antibacterial activities against bacteria and by increasing the diameters of inhibition where the diameters zones of inhibition of the parent complex (1) the bacteria under study are (13,10, 11 and 12) mm, respectively, and increased diameters of inhibition when adding copper chloride (complex 2) to become (16, 12, 13 and 13) mm, as recorded over an increase in the diameters of inhibition against the bacteria under study when adding chloride Lanthanum (complex 3) was hailed diameters of inhibition (17, 14, 15 and 14) mm, respectively, as shown in Tables 1-3. These results coincided with findings ( LIN Jianyuan, *et al*., 2012) Which showed Antibacterial activity tests show that in the case of antibacterial activity,the lanthanum-rutin complex is superior to the rare earth lanthanum(Ⅲ) ion and rutin ligand.The fabric finished with the complex is rendered good antibacterial activity **[ 27 ]** .

The results showed Staphylococcus aureus bacteria (positive for the dye gram) more types of bacteria are most affected by the (response) to the effect of complex that used where the highest recorded diameters inhibition compared with other types of bacteria(negative bacteria for gram) that showed influential variably , Where the diameters of inhibition zone of bacteria staph.aureus (13, 16, 17) mm for complexes under study respectively, might be structural composition of bacterial wall lack the bacteria trigger a gram to the layer of the external membrane permeability material to make into the cell than negative bacteria for a gram **[ 28 ]** .

## 4. Conclusion

By the results that appeared to have conclude that the addition of chloride each of copper and lanthanum to the parent complex led to increased antibacterial activities against bacteria and by increasing the diameters of inhibition where the diameters zones of inhibition of the parent complex (1) , the Lanthanum complex is more effective against almost all antibacterial tested.

Furthermore The *staphylococcus aureus* bacteria was more types of bacteria sensitive to the effect of the complex studied .

## 5. References:

1. Benbow M.( 2010), Wound swabs and chronic wounds. Practice Nurse; 39(9):27–30 .

2. Edwards R & Harding KG.( 2004) Bacteria and wound healing. Curr Opin Infect Dis; 17:91–96.

3.Angel DE, Lloyd P, Carville K & Santamaria N.(2011),The clinical efficacy of two semi-quantitative wound swabbing techniques in identifying the causative organism(s). Int Wound J; 8:176–185.

4. Bowler PG, Duerden BI, Amstrong DC.( 2001), Wound microbiology and associated approaches to wound management. Clin Microbiol Rev.;14:244-269.

5. Dionigi R, Rovera F, Dionigi G, Imperator A, Ferrari A, Dionigi R. et al.( 2001) , Risk factors in surgery. J Chemother. 13:6-11.
6. Iroha IR, Amadi ES, Orji JO. Esimone CO.( 2008), In vitro evaluation of the activity of colloidal silver concentrate against Pseudomonas aeruginosa isolated from postoperative wound infection. Sci Res Essay.; 3(5):209-211.
7. Akinjogunla OJ, Adegoke AA, Mboto CI, Chukwudebelu IC. Udokang IP.( 2009) , Bacteriology of automobile accident wounds infection. Int J Med Med Sci.;1(2):23-27.
8. Alexander MF.( 1994) ,Wound infection. In: Alexander MF, Fawcett JN, Runciman PJ, editors. Nursing Practice Hospital and Home, the Adult New York: Churchill. Livingstone;. p. 703.
9. Trilla A.( 1994), Epidemiology of nosoconial infections in adult intensive care units. Intensive care units. Intensive Care Med.;20(3):1-4.
10. Yah SC, Enabulele IO. Eghafona NO.( 2004), Bacteriological studies on infected Kerosene burn wounds in Benin City, Nigeria. J Biomed Invest.;2(1):4-9.
11. Yah SC, Enabulele IO, Eghafona NO. Udemezue OO.( 2004), Prevalence of Pseudomonas in burn wounds at the University of Benin Teaching Hospital, Benin City, Nigeria.J Clin Exp Anat.;3(1):12-15.
12. Amelski R. Barczynski M.( 2002), Wound infection. Aresteziol reanimatol;14:91-93.
13. Enweani IB, Esumeh FI, Akpe RA, Tatfeng MY. Isibor JO.( 2003),Bacteria associated with post operative wounds. J Contem Issues.;1:183-188.
14. Kaplan NM, Smadi AA, Taani MI. El-Qudah MA.( 2003),Microbiology of wound infection after caesaria section. Est Meditter Health J.;9(5-6):10681074.
15. Isibor JO, Oseni A, Eyaufe A, Osagie R. Turay A.( 2008),Incidence of aerobic bacteria and Candida albicas in post operative wound infections. Afr J Microbiol Res.;2:288-291.
16. Islam, M.A., Farooque, M.A., Bodruddoza, M.A.K. Mosadik, M.A. and Alam M.A. (2002). Antimicrobial and Toxological Studies of Mixed Ligand Transition Metal Complexes of schiff bases, Bio. Sci. 2(12): 797-799.
17. Part, W.B. and Ruddon, W. (1979). The anticancer drugs. 251-254.
18. Zakaria C.M., Farooque, M.A., Islam, M.R. and biswas M.H. (2000). Antimicrobial screening of ferrocene derivative compounds, Oriental Journal of Chemistry. 16(1):85-90.
19. Shulman, A., Dwyer, F.P., Dwyer, F.P., dayer and mellor D.P.( 1967). Chelating agents and metal chelates. Academic press New York 303.
20. Curtis, N.F. (1968). Coord. Chem. Rev. 3:3
21. Sadler, P.J., Nast, M. and Narayanan VL. (1983). The design of metal complex as anticancer drugs. Martius-Nijhoff, Bostom.
22. Tipton, L.H., Cook, H.M., Steiner r.L. and Boyle C.A.( 1979). Trace elements in human tissues. Health Physics, Pergmon Prss, lx 89.
23. Klyman, D.L., Scovil, J.P. and Bastoerich J.F., Manson, C.J. (1979). J. Med. Chem. 22 : 855.
24. Nofal, Z. M., El-Zahar M. I. and Abd El-Karim S. S.( 2000) .Novel Coumarin Derivatives with Expected Biological Activity. J. Molecules. , 5, 99-113.
25. Bernadette S. Creaven, Denise A. Egan, Kevin Kavanagh, Malachy McCann , Andy Noble, Bhumika Thati and Maureen Walsh.( 2006) ,Synthesis, characterization and antimicrobial activity of a series of substituted coumarin-3-carboxylatosilver(I) complexes . Inorganica Chimica Acta359 : 3976–3984 .
26. Holt ,JG. ( Ed).(1986), Bergey's manual of systematic bacteriology, 2,4 t h ed. S.T. Williams and M.E. Sharpe, Baltimore, Md: Williams and Williams.
27. LIN Jianyuan,LUO Linfeng,CHEN Liang,YU Hongzhe .(2012),Synthesis,characterization and antibacterial activity of rare earth lanthanum-rutin complex . College of Biomedical and Environmental Science,Zhejiang Wanli University,Ningbo,Zhejiang 315100,China .
28. Myrvick, N. and Weiser, S. (1988). Fundamentals of Medical Bacteriology and Mycology . 2nd ed. Lea and Febiger. Philadelphia.

**Table 1.** Antibacterial activity of the complex **1** (**ethyl 2-oxo-2H-chromene-3-carboxylate**)

| Name of bacteria | Diameter of inhibition zone of bacteria in mm | Cefoxitin 30µg/disc |
|---|---|---|
| *Staphylococcus aureus ( +ve )* | 13 | 16 |
| *Escherichia coli (-ve)* | 10 | 18 |
| *Pseudomonas aeruginosa ( -ve)* | 11 | 18 |
| *Proteus mirabilis ( -ve )* | 12 | 17 |

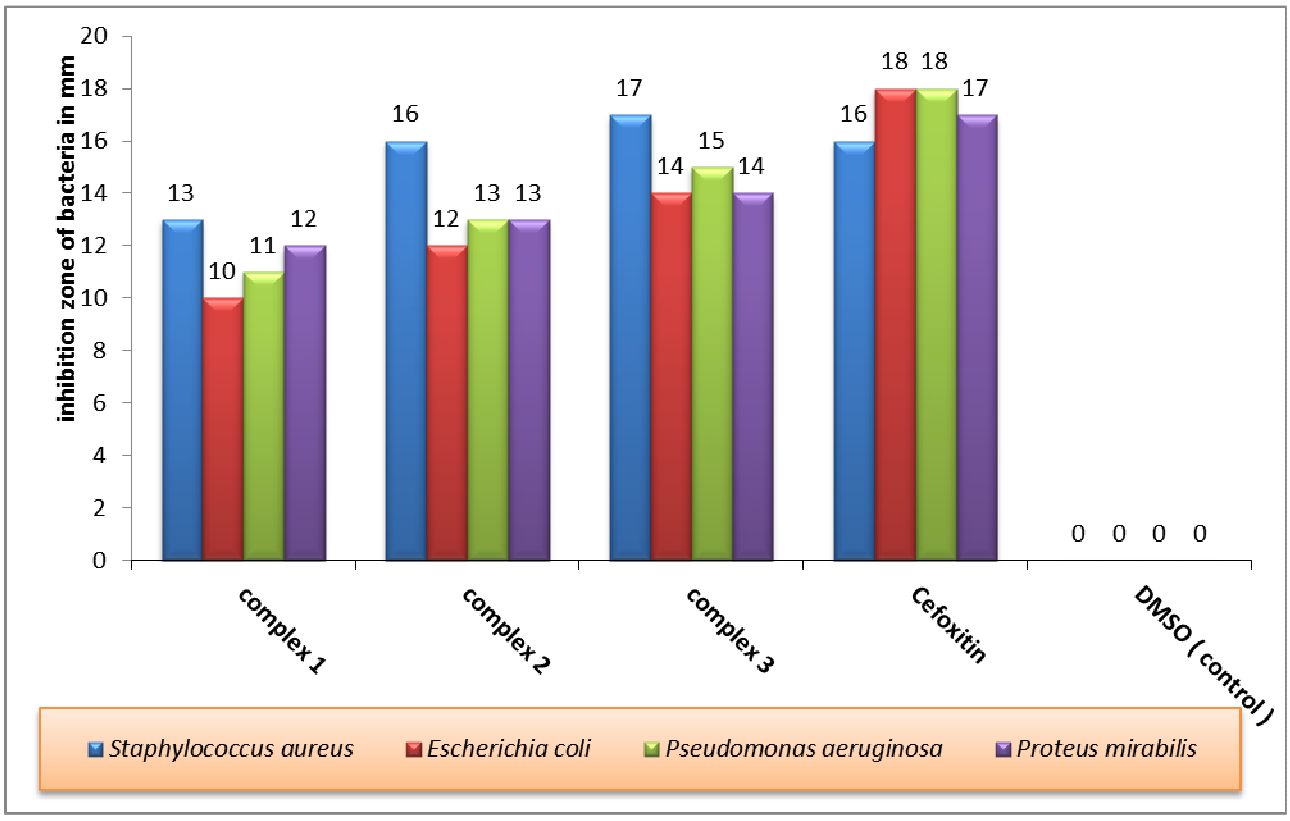
**Table 2.** Antibacterial activity of the complex **2 [ Cu(ethyl 2-oxo-2H-chromene-3-carboxylate)$_2$Cl$_2$]**

| Name of bacteria | Diameter of inhibition zone of bacteria in mm | Cefoxitin 30µg/disc |
|---|---|---|
| *Staphylococcus aureus ( +ve )* | 16 | 16 |
| *Escherichia coli (-ve)* | 12 | 18 |
| *Pseudomonas aeruginosa ( -ve)* | 13 | 18 |
| *Proteus mirabilis ( -ve )* | 13 | 17 |

**Table 3.** Antibacterial activity of the complex **3 [ La(ethyl 2-oxo-2H-chromene-3-carboxylate)$_2$Cl$_2$]Cl**

| Name of bacteria | Diameter of inhibition zone of bacteria in mm | Cefoxitin 30µg/disc |
|---|---|---|
| *Staphylococcus aureus ( +ve )* | 17 | 16 |
| *Escherichia coli (-ve)* | 14 | 18 |
| *Pseudomonas aeruginosa ( -ve)* | 15 | 18 |
| *Proteus mirabilis ( -ve )* | 14 | 17 |

**Figure 1. Antibacterial effect of the studied complexes.**



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