The Antibacterial Activity of Dimethyl Sulfoxide (DMSO) with and without of Some Ligand Complexes of the Transitional Metal Ions of Ethyl Coumarin against Bacteria Isolate from Burn and Wound Infection

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Abstract
Antibacterial activities (in vitro study) of Dimethylsulfoxide (DMSO) with and without of some ligand complexes of the transitional metal ions of ethyl coumarin were carried out with the help of agar well diffusion method technique against four human pathogenic bacteria isolation from burn and wound infections. Observed through the results and the presence of DMSO obvious effect on the bacterial species under study. When melting ligand complexes of the transitional metal ions of ethyl coumarin in DMSO and tested on bacteria see a significant increase in the diameters of the inhibition of the growth of bacteria, compared with the use of DMSO alone, it has observed that the complex 3and 4 was more active than the others. The complexes 1,2,5,6 also has showed substantial antimicrobial activity. Furthermore The staphylococcus aureus bacteria was more types of bacteria sensitive to the effect of the complex studied.

Keywords: Antimicrobial activities, Dimethylsulfoxide (DMSO), human pathogenic bacteria, burn & wound infections.

1. Introduction
The skin, one of the largest organs in the body, performs numerous vital functions, including fluid homeostasis, thermoregulation, immunologic functions, neurosensory functions, and metabolic functions (eg, vitamin D). The skin also provides primary protection against infection by acting as a physical barrier. When this barrier is damaged, pathogens have a direct route to infiltrate the body, possibly resulting in infection [1].

Burn wound infection is problematic because it delays healing, encourages scarring and may result in bacteremia, sepsis or multiple-organ dysfunction syndrome (a.k.a. organ failure) whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention [2].

Bacteria and fungi are the most common pathogens of burn wounds. These microbes form multispecies biofilms on burn wounds within 48 – 72 hours of injury [1]. Organisms originate from the patient’s own skin, gut and respiratory flora, as well as from contact with contaminated health care environments and workers [1,3]. Gram-positive bacteria are some of the first to colonize burns, followed quickly by gram-negative. Fungal infection tends to occur in the later stages after the majority of bacteria have been eliminated by topical antibiotics [1]. Two bacterial species, methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa will be examined in depth in this page as they are two of the most prevalent infective agents. These two species have proven particularly difficult to treat because they possess a large number of virulence factors and antimicrobial resistance genes.

The risk of infection in burns is well-known. In recent decades, the antimicrobial resistance of bacteria isolated from burn patients has increased. For this reason, a retrospective study was conducted at Van Training and Research Hospital to analyze the bacterial isolates from the wounds of patients admitted to the Burn Unit and to determine the susceptibility patterns of the commonly cultured organisms over a 3-year period, January 2009 to December 2011. A total of 250 microorganisms were isolated from burn wounds of 179 patients. Our results revealed that the most frequent isolate was Acinetobacter baumannii (23.6%), Pseudomonas aeruginosa (12%), Staphylococcus aureus (11.2%), Escherichia coli (10%) respectively [4].

Dimethyl sulfoxide (DMSO) is an organ sulfur compound with the formula (CH3)2SO. This colorless liquid is an important polar aprotic solvent that dissolves both polar and no polar compounds and is miscible in a wide range of organic solvents as well as water [5]. It penetrates the skin very readily and has the unusual property that many individuals perceive a garlic-like taste in the mouth after contact of DMSO with the skin [6].

Use of DMSO in medicine dates from around 1963, when an Oregon Health & Science University Medical School team, headed by Stanley Jacob, discovered it could penetrate the skin and other membranes without damaging them and could carry other compounds into a biological system. In medicine, DMSO is predominantly used as a topical analgesic, a vehicle for topical application of pharmaceuticals, as an anti-inflammatory, and an antioxidant [7]. Because DMSO increases the rate of absorption of some compounds through organic tissues, including skin, it is used in some transdermal drug delivery systems. Its effect may be enhanced with the addition of EDTA. It is frequently compounded with antifungal medications, enabling them to
penetrate not just skin but also toe and fingernails [8].

The pharmacologic effects of DMSO are diverse: it traps free radical hydroxide and its metabolite, dimethyl sulfide (DMS) traps free radical oxygen. It appears that these actions help to explain some of the anti-inflammatory, cryopreservative, antiischemic, and radioprotective qualities of DMSO. Dimethyl sulfoxide will easily penetrate the skin. It also serves as a carrier agent in promoting the percutaneous absorption of other compounds (including drugs and toxins) that normally would not penetrate. Drugs such as insulin, heparin, phenylbutazone, and sulfonamides may all be absorbed systemically when mixed with DMSO and applied to the skin. The anti-inflammatory/analgesic properties of DMSO have been thoroughly investigated. DMSO appears to be more effective an anti-inflammatory agent when used for acute inflammation versus chronic inflammatory conditions. The analgesic effects of DMSO have been compared to that produced by narcotic analgesics and is efficacious for both acute and chronic musculoskeletal pain [9].

DMSO is often used as a cream or ointment applied to the skin to reduce pain, decrease swelling, treat autoimmune diseases such as arthritis, and promote healing in wounds and burns [10].

Effective skin antisepsis is of central importance in the prevention of wound infections, colonization of medical devices, and nosocomial transmission of microorganisms. Current antiseptics have a suboptimal efficacy resulting in substantial infectious morbidity, mortality, and increased health care costs. Here, we introduce an in vitro method for antiseptic testing and a novel alcohol-based antiseptic containing 4 to 5% of the polar aprotic solvent dimethyl sulfoxide (DMSO). The DMSO-containing antiseptic resulted in a 1- to 2-log enhanced killing of Staphylococcus epidermidis and other microbes in vitro compared to the same antiseptic without DMSO. In a prospective clinical validation, blood culture contamination rates were reduced from 3.04% for 70% isopropanol–1% iodine (control antiseptic) to 1.04% for 70% isopropanol–1% iodine–5% DMSO ($P < 0.01$). Our results predict that improved skin antisepsis is possible using new formulations of antiseptics containing strongly polarized but nonionizing (polar aprotic) solvents [11].

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 cytotoxic potential of coumarins complexes 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 [12].

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-Mustansiriya. 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 burn and wound infections, the organisms were identified by standard microbiological techniques including colonial characteristics, morphological characteristics and biochemical characteristics [13, 14].

2.2 Chemical Synthesis of ligands complex (complex 1, 2, 3, 4, 5 and 6)

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.
carried out with the help of agar well diffusion method technique. Each well contained 10 µL volume of
(0.371 g, 1 mmole) of chloride 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.

2.2.3 complex 4,5,6: dichloro bis (di ethyl 2-oxo-2H-chromene-3-carboxylate) Zinc (II) (4), bis (di ethyl 2-oxo-2H-chromene-3-carboxylate) die quo Cadmium (II) chloride (5), bis (di ethyl 2-oxo-2H-chromene-3-carboxylate) die quo Mercury (II) chloride (6).

Added (0.2 grams, 0.32 grams and 0.38 grams, 1 mmole) of chloride each of Zinc, Cadmium and Mercury respectively and dissolved the lowest amount of ethanol absolute to (0.218 g, 1 mmoles) of the compound 1 dissolved in 15 mL of solvent was then escalate the combination of each complex for two hours later filtered and re-crystallized in hot absolute ethanol solvent and then dried residue complexes and calculated weights.

2.3 Antibacterial activity

The Dimethyl sulfoxide (DMSO) and complexes used to evaluate the antibacterial activity against staphylococcus aureus, Escherichia coli, pseudomonas aeruginosa and Proteus mirabilis. The activities were carried out with the help of agar well diffusion method technique. Each well contained a 10 µL volume of compound and it was placed on bacteria inoculated plates. The growth inhibition results were compared with standard antibiotic Cefoxitin 30 µg/disc which were used as control.

In this method, pure isolate of 24hrs growth (containing 1.5 × 10⁸ cells / ml ) was cultured in Muller-Hinton Agar plate by using sterile swab so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 8.0 mm was used to bore wells in each agar plates. A 10 µL volume of each compound that Concentration 200 µg / ml was applied by micropipette in the wells into Muller-Hinton Agar plate. standard antibiotic Cefoxitin 30 µg/disc use as control. The plates were allowed to stand for 1 h or more for diffusion to take place and then incubated at 37°C for 24hrs. The antimicrobial activity of each compound was recorded by measuring the inhibition zone around the wells [15].

3. Results and Discussion

Antibacterial activities (in vitro study) of Dimethylsulfoxide (DMSO) with and without of some ligand complexes of the transitional metal ions of ethyl coumarin these complexes were tested in the present studies and results are presented in Tables 2. The antimicrobial activity of the (DMSO) and complex 1, 2,3,4,5 and 6 were determined at the concentration of 200 µg/ml against a series of Gram positive and Gram negative pathogenic organisms isolated from burn and wound infections. From the zone of inhibition, by using agar well diffusion method technique, the bacterial isolates were diagnosed according to their morphological, colonial and biochemical characteristics results are presented in Tables 1.

Observed through the results and the presence of DMSO obvious effect on the bacterial species under study, where the diameters of inhibition zones were (28,25, 21 and 20) mm, respectively. These results agreed with the study conducted by H. BASCH AND HH GADEBUSCH (1968) which emphasized that the DMSO exerted a marked inhibitory effect on a wide range of bacteria and fungi, including one parasite, at concentrations likely to be encountered in antimicrobial testing programs in industry [16]. And Agreed with a nether study Conducted by Muhammad Akram Rdhawat (2006) the present study aimed at determination of the effect of different concentration of DMSO (0.125 to 10 %) on growth of dermatophytes by agar diffusion method, there was no growth of fungi in 10% DMSO between 1.25 an 5% there was a rather linear dose-related inhibitory effect on the growth, significantly different from the control, and below 1% there was variable effect among the species [17].

When melting ligand complexes of the transitional metal ions of ethyl coumarin in DMSO and tested on bacteria observed a significant increase in the diameters of the inhibition of the growth of bacteria, compared with the use of DMSO alone, it has observed that the complex 3 and 4 was more active than the others. The complex 1, 2, 5, 6 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 melting each ligand complexes of the transitional metal ions of ethyl coumarin in DMSO led to increased antibacterial activities against bacteria and by increasing the diameters of inhibition where the diameters zones of inhibition of the parent DMSO only the bacteria under study are (28,25, 21 and 20) mm, respectively, and increased diameters of inhibition when adding DMSO to Compound 1 to become (36, 32, 35 and 35) mm, as recorded over an increase in the diameters of inhibition against the bacteria under study when adding DMSO to Compound 3 was hailed diameters of inhibition (42, 39, 38 and 38) mm, respectively, as shown in Tables 2. These results coincided with findings LIN Jianyuan (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 (La) ion and rutin ligand. The fabric finished with the complex is rendered.
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 (28, 36, 38, 42, 42, 40 and 42) 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.

4. Conclusion

By the results that appeared to have conclude that melting each ligand complexes of the transitional metal ions of ethyl coumarin in DMSO led to increased antibacterial activities against bacteria and by increasing the diameters of inhibition compared with the use of DMSO alone, As well as infer that the complex 3 and 4 (Lanthanum and Zinc 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

Table 1. Morphological and Biochemical tests that used to identify isolated bacteria from burn and wound infections.

<table>
<thead>
<tr>
<th>Test</th>
<th>E. coli</th>
<th>P. mirabilis</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram's stain</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Oxidase test</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>Catalase test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Indole test</td>
<td>+</td>
<td>-</td>
<td>*</td>
<td>*</td>
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<tr>
<td>Methyl red test</td>
<td>+</td>
<td>+</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Voges-Proskauer test</td>
<td>-</td>
<td>/+</td>
<td>*</td>
<td>*</td>
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<tr>
<td>Citrate utilization</td>
<td>-</td>
<td>/+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>Lactose fermentation</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Urease test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Triple sugar iron agar &amp; H2S production</td>
<td>A/A -</td>
<td>Alk/A +</td>
<td>Alk/Alk -</td>
<td>*</td>
</tr>
<tr>
<td>Motility test</td>
<td>/+-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Growth at 42°C</td>
<td>*</td>
<td>*</td>
<td>+</td>
<td>*</td>
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<tr>
<td>Mannitol fermentation</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>+</td>
</tr>
<tr>
<td>Coagulase test</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>+</td>
</tr>
</tbody>
</table>

(+): positive, (-): negative, (*) not tested (A/A -): acidic/acidic no H2S production (Alk/Alk -): alkaline/alkaline no H2S production (Alk/A +): alkaline/acidic with H2S Production
Table 2. Antibacterial activity of the (DMSO) with and without of some ligand complexes.

<table>
<thead>
<tr>
<th>complexes</th>
<th>Bacteria</th>
<th>Staphylococcus aureus (+ve)</th>
<th>Escherichia coli (-ve)</th>
<th>Pseudomonas aeruginosa (-ve)</th>
<th>Proteus mirabilis (-ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO only</td>
<td></td>
<td>28</td>
<td>25</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>complexes with DMSO</td>
<td>Diameter of inhibition zone of bacteria in mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>complex 1 + DMSO</td>
<td></td>
<td>36</td>
<td>32</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>complex 2 + DMSO</td>
<td></td>
<td>38</td>
<td>34</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>complex 3 + DMSO</td>
<td></td>
<td>42</td>
<td>39</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>complex 4 + DMSO</td>
<td></td>
<td>42</td>
<td>36</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>complex 5 + DMSO</td>
<td></td>
<td>40</td>
<td>35</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>complex 6 + DMSO</td>
<td></td>
<td>42</td>
<td>38</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>17</td>
</tr>
</tbody>
</table>

complex 1 = ethyl 2-oxo-2H-chromene-3-carboxylate (L); complex 2 = [Cu(L)₂Cl₂]; complex 3 = [La(L)₂Cl₂]Cl; complex 4 = [Zn(L)Cl₂]; complex 5 = [Cd(L)(H₂O)₂]Cl₂; complex 6 = [Hg(L)(H₂O)₂]Cl₂; DMSO = Dimethylsulfoxide; control = Cefoxitin (FOX).

Figure 1. Antibacterial effect of the studied complexes.
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