

**The microbial effect of concentrations some minerals on the growth of bacteria (Staphylococcus and Klebsiella) isolated from some hospital rooms in Derna and Al-Bayda**

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**Abstract:**

This study aimed to isolate and identify 110 samples from some rooms of different departments in Derna and Al-Bayda hospitals in order to determine the bacterial species that are likely to be present inside the hospitals. Some known microbiological methods were used to identify the isolated bacterial species, such as the spectrophotometer, the minimum inhibitory concentration (MIC), and the agar diffusion method. Concentrations of some elements (nickel, iron, copper) were used as inhibitors for the isolated bacterial species. Statistical analysis was performed using Tukey analysis, MINITAB program, version 2021. The results showed that the most isolated bacterial species were Staphylococcus at a rate of 20% and Klebsiella at a rate of 8.9%. The effectiveness of the concentrations of some of the elements under study on the growth of the isolated bacterial species was studied. The results showed that the nickel element was the most effective inhibitor at a concentration of 100% among the elements, followed by copper at a concentration of 100%, and the least effective element was iron on Staphylococcus bacteria, while on Gram-negative bacteria Klebsiella Copper was the most effective element at 100% concentration, followed by nickel, and iron had no effective effect on Klebsiella.

**Keywords:** Bacterial isolation; Staphylococcus; Klebsiella; Minimum Inhibitory Concentration (MIC), Antimicrobial resistance, Hospital-acquired infections, Phytochemical analysis.

## 1. INTRODUCTION

Nosocomial infection can be caused by any organism, yet certain organisms are more likely to be the source of hospital-acquired infections (Hu et al., 2021; Grimes et al., 1982).

The results of a study done in Europe show that the prevalence of HAIs Numerous bacteria can cause nosocomial infections, and each one can result in infection in medical environments. Bacteria cause Ninety percent of illnesses, with less of an influence from fungus. Hospital-acquired infections are typically caused by a variety of agents, such as Streptococcus species, Acinetobacter species, Enterococci, Pseudomonas aeruginosa coagulase-negative Staphylococci, Staphylococcus aureus Bacillus cereus Legionella, and members of Klebsiella pneumonia, and the numerous species of Staphylococcus bacteria, Staphylococcus aureus considered a very important pathogen responsible for many infections acquired in healthcare (Laham et al., 2015).

It is more than just that we are more likely to contract S. aureus infections in communities. Not only can S. aureus infect surface tissues, but it can also infect deep tissues and local abscess lesions. Food poisoning from ingesting enterotoxins is one of the toxin-mediated disorders caused by S. aureus, whereas exfoliate toxins produce staphylococcal scalded skin syndrome (Laham et al., 2015).

K. pneumoniae is the seventh major pathogen in healthcare settings, accounting for three to seven percent of hospital-acquired bacterial infections. It belongs to the Enterobacteriaceae family and is an opportunistic, Gram-negative bacillus. It typically colonizes the skin, throat, and digestive tract. It is associated with illnesses like septicemia, wound infections, pneumonia, and newborn septicemia. One of the main factors contributing to nosocomial infection consequences is resistance to  $\beta$ -lactam medicines. K. pneumoniae and E. coli are two of the microorganisms that are resistant to medications that include  $\beta$ -lactamase. Third- and fourth-generation cephalosporins exhibit K. pneumoniae. (Vandenesch et al., 2012).

It most likely plays a part in maintaining the stability of RNA structure. Divalent nickel works in tandem with the enzyme Glyoxalase I to facilitate the breakdown of methylglycol, lactate, and water. An octahedral high-spin Ni (II) complex called acereductonedioxygenase catalyzes the breakdown of the peroxo intermediate. Coordination connections can be formed between metal ions (Chivers and Laitinen, 2015).

There is a clear correlation between the rise in infectious disease-related fatalities in Africa and bacterial multi-antibiotic resistance. The primary cause of this problem is the dearth of potent antibacterial drugs. Recent research has focused mostly on the synthesis of nickel complexes with ligands due to their biological properties (Wilfred et al., 2012).

There is no doubt that developing new antibacterial agents through a range of methods is a pressing medical need (Petzold and Al-Hashimi, 2011, Adams et al., 2018).

The development of the latest nickel-based pharmaceuticals has been greatly aided by nickel's ability to increase the inhibitory power of chemotherapeutic medications. Coordination has been found to improve the efficacy of a range of medical therapies (Wilfred et al., 2012).

In the past few years, research has been conducted to create the metal copper, which has been shown to have self-sanitizing properties that prevent human infections from surviving exposure to copper or copper alloy surfaces for any appreciable amount of time. There is a significant reason for concern in the current pandemic context because this feature is not observed with other common surface materials such as plastic, stainless steel, or aluminum (Olivares and Uauy, 1996; Carson et al., 2007; Brewer, 2003).

Three hundred different copper surfaces were registered with the US Environmental Protection Agency (USEPA) in 2008 as a result of testing conducted in an independent microbiology laboratory about the efficacy of copper surfaces (Grass et al., 2011; Michels et al., 2008). The following is stated in the registration: "When routinely cleaned, the antimicrobial coppermetals have antimicrobial qualities that have been understood for many years (Ogunniran et al., 2008) .

In addition, the evaluation of their combination with antibiotics has been done in multiple trials. For instance, research has demonstrated that  $\beta$ -lactams combined with silver or zinc oxide nanoparticles have excellent results (Alekhshun and Levy, 2007) .

In the form of a metal complex, iron is the most prevalent transition element in the human body and a potentially effective antibacterial agent. Iron can affect bacterial cells by inducing oxidative stress, blocking respiration and ATP synthesis, increasing cell hydrophobicity, and facilitating penetration over the cell wall. Consequently, combination treatments provide a number of benefits, including a decreased risk of developing (Saranya et al., 2020; Claudel et al., 2020).

## 2. Materials and methods

### 2.1. Study locations

This study was conducted at Al-Bayda Medical Center in Al-Bayda and Al-Wehda Hospital in Derna, (Libya), between (October 2022-December 2023), Al-bayda and Derna are cities located in the northern eastern region in Libya as shown in figure (1).



Figure: (1). Map of Libya with relative location of Derna and Al-Bayda hospitals with local samples.

### 2.2. Isolation and identification

#### 2.2.1. Sampling:

The samples were collected from two different hospitals (Al-Bayda Medical Center and Al.wahda Derna) from some departments, Benches and rooms. The samples were selected randomly, where 112 samples were taken from pediatric Department, incubators, some rooms, sterilization devices, monitoring devices, cauterization devices, emergency rooms, surgical department rooms, doors, handles and ventilators .

For Al-Bayda Hospital, the samples were collected from women's Department only, which consisted of cauterization devices, monitoring devices, emergency carts, sterilization devices and seats.

### **2.2.2. Isolation**

The samples were isolated from the selected collection sites. A conventional Nutrient Broth bacterial medium was prepared according to the manufacturer's instructions on the package. Then the medium was sterilized in sterile tubes under sterile conditions. Swabs were taken from the locations, placed in test tubes under sterile conditions, and transferred to the laboratory to conduct the known microbiological tests (Benson, 1990).

### **2.2.3. Identification**

Bacteria were identified using conventional diagnostic microbiological laboratory techniques as well as morphological appearance. They were cultured on selective media to determine the growth type of each bacterial isolate. Blood agar, MacConkey agar, mannitol agar, NB and NA nutrient agar were used. They were incubated for 24 h at 37°C. Gram stain was then performed to determine whether they were Gram-positive or Gram-negative. Biochemical tests, including catalase and oxidase tests, were performed (Benson, 1990).

## **2.3. Antimicrobial investigation**

### **2.3.1. Spectrophotometric method**

The spectrophotometric method which carried out in this study was carried by measured the mixture of isolated bacteria with the suggested metal ion solutions .

A suspension of  $1.5 \times 10^8$  CFU/ml (Farland method, 1907) was prepared for bacterial species of (Klebsiella, pseudomonas and S.aureus). where 1 ml of the bacterial suspension and 1 ml of the element or plant extract used in this study were taken, which are (Different concentrations of iron, copper and nickel Solutions) and extracts of the dodder plant and 1 ml of sterile nutrient broth and incubated at a temperature of 37 C° for 24 hours. Then, a spectrophotometer was used to estimate the turbidity values, where the, the optical density (OD) of the designated broth media was measured using a spectrophotometer set at a wavelength of 600 nm to improve accuracy, then slightly diluted before taking the readings. These measurements were then repeated throughout the experiment at selected time points (Lin et al., 1989). Different concentrations of (5, 10 ,25,75,50 and 100 ppm) for metal ions in addition to the extract plant Dodder and minerals at all tested species of organisms (Wadhvani et al., 2019).

### **2.3.2 Agar wellpropagation techniques**

Antibacterial activity was confirmed using the agar diffusion method and using three bacterial colonies, namely Klebsiella pseudomonas and S.aureus, using nutrient agar (NA) and sterile cotton swabs. A bacterial suspension was prepared using the MC Farland method. The suspension was distributed on the plates, then using a sterile drill to create a bacterial well with a diameter of 4 mm in each plate; concentrations of mineral elements and different extracts Dodder plant were added at a rate of about 100 microliters. After 24 hours in the Incubation, the results were read using a ruler by measuring the diameter of the Inhibition zone (Mahmoudi et al., 2011).

### **2.2.4. Minimum inhibitory concentration (MIC) test**

Different concentrations of metal elements (Iron, Copper and Nickel) and extracts of the plant Dodder according to the method of the National Committee for Clinical Laboratory Standards (NCCLS,2000), In this study each one of the concentrations 25, 50, 75 and 100 ppm to the Nutrient Agar (NA) medium at 50°C. The suspension was distributed on plates and then left for few minutes to dry. The plant were 24 hours at 37°C. A bacterial suspension was prepared without any antimicrobial agents. It is used to

determine the actual growth of bacteria (Ericsson et al., 1960). The results were recorded as the presence or absence of growth.

### 3. Results

The obtained results were described according to the applied methods in this study (spectrophotometer and diffusion method).

#### 3.1. Isolation and identification bacteria species:

Colonies appeared on transitional media and were confirmed by biochemical tests and Gram stain, where under the microscope a large, spherical, bright red colony appeared, non-motile, Gram-negative *Klebsiella*, while a blue colony appeared under the microscope, spherical, resembling a bunch of grapes, irregular balls. The result of the coagulase test showed blood clotting and the colonies appeared yellow on Mannitol salt agar medium, due to its ability to completely decompose red blood cells, anaerobic.

The results of isolation bacteria for the 110 samples which were used in this study showed that the species of bacteria *S.aureus* 20% and *Klebsiella* 8.9%, as shown in Table (1).

Table: (1). Distribution values of bacterial isolates at sampling sites.

Isolation bacteria	Average
<b>Staphylococcus aureus</b>	<b>20.719b±.1239</b>
<b>klebsiella</b>	<b>8.944c±7.87</b>
<b>Tukey's</b>	<b>0.2652 ***</b>

#### 3.2. Spectrophotometer method

##### 3.2.1. Effect of metal concentrations and Dodder plant extract on isolated bacteria inhibition by spectrophotometric method

The high spectrometer was set to measure the transmittance 600nm which refer to the optical density at a wavelength of 600nm transmittance measurement was taken without adding coefficients at 100% and when adding substances at zero high turbidity little or no light transmittance occurs indicating high bacterial growth because the growth intercepts the light and it is absorbed instead of going into The other way and transmittance tells us how light passes through the sample that contains a large number of cells and the transmittance reading will be lower than the sample which contains a lower number of cells gives a higher reading and this is what we indicated in concentration 100 where the reading was higher and the number of microbial cells was lower.

Showed the effect of different concentrations of metal ions (50, 75 and 100%) showed that the low concentrations of metal ion solutions (5, 10, 25%) were the readings respectively iron (5, 10, and 25%) (0.2, 4.5, and 2.4) copper (0.9, 4.6, and 2.2) nickel (1.2, 2.3, and 1.7) dodder (0.1, 0.5 and 0.9) did not show any bacterial activity on the other side the metal concentrations of 50, 75, and 100% recorded different antibacterial activities (6.7, 4.9, 2.1, and 35.96 nm) respectively the metal concentration of 100% gave high antibacterial permeability of (35.96 nm) the results were illustrated Tables (2).

For the Nickel concentrations (50, 75 and 100 %) effect, the results recorded that, the high concentration of 100 % gave antibacterial activities of (87.133, 16.2 and 96.76 nm) on *Staphylococcus aureus*, *klebsieia* species, respectively. While the Nickel concentrations of (75 %) recorded inhibition, *Bacteria* values of (4, 20.66 and 14.07 nm) on *Staphylococcus aureus*, *klebsieia* species, respectively. On the other side, the Nickel concentrations of (50 %) recorded lower antibacterial values of (8.06, 1.1 and 10.20

nm), on *Staphylococcus aureus*, *klebsieia* species, respectively, the same manner was recorded for the effect of Iron solution concentrations on the selected bacteria species.

Also, the concentrations of copper solutions of (50, 75 and 100 %) recorded antibacterial activities on the studied specie's (*Staphylococcus aureus*, *klebsieia*), where the inhibition values (nm) were as following: (49, 27 and 44 nm), (15, 7 and 11 nm) and (7, 2, and 9 nm) on the above concentrations and bacteria species, respectively.

Table: (2). Effect of metals concentrations antibacterial activity.

Concentrations	Average (nm)
100%	35.961a±29.64
75%	9.217b±5.702
50%	6.747c±4.080
Tukey's	0.4593 ***

The results of the first experiment containing three treatments, the first factor isolates used in the study, the second factor is elements, minerals, the third factor is concentrations at a significant level of 0.001, the coefficient of estimation of the difference was 99.65%, evidence of the accuracy of the experiment according to the statistical analysis of the normal distribution using Completely Randomized Design (CRD) and using the Turkey's test using the MINITAB program by version 2021, Among the metal concentrations the one with the highest permeability was nickel at a concentration of 100% and copper at a concentration of 100%, then iron permeable according to the Tukey's test, which showed a significant difference between the averages, which were (a), (b), and then (c), as shown in Table (3).

Table: (3). The antibacterial activity of different concentrations at metals (OD. Nm)

Isolation Concentrations	S. aureus			Klebsiella		
	100%	75%	50%	100%	75%	50%
Ni	87.133 b	4 o	8.067	16.2	20.667 f	1.1 r
Fe	32.733 d	3.2 o	1.233 q	2.733	6.067	6.6
Cu	49.067 c	15.767 f	7 k	27.267 e	7.733	2.333
Tukey's	0.9185***					

### 3.3. Agar diffusion method

#### Effect of metal concentrations on isolated bacteria inhibition by Agar diffusion method

The solutions of metals (Fe, Ni and Cu) for concentrations of (50, 75 and 100 %) showed small of antibacterial activities compared with the values of those values obtained by spectrophotometric methods. In addition, the concentrations of (Fe) did not show any antibacterial activities. The concentrations (100 %) of Nickel solutions showed of (11, 9 and 11 mm), while the isolated bacteria *S.aureus* and *Klebsiella*, whereas the concentrations at (75 %) gave inhibition values. In addition, for the concentrations of (10, 8 and 10 mm) and for the Copper concentrations of the same concentrations of

(50, 75 and 100 %) for copper recorded values (11, 9 and 8) for 100 % against S.aureus , in addition to values of (10 ,7 and 6 mm) for (75 %) against.

Table: (4). Effect antibacterial activity different metals concentrations and isolation by (Agar diffusion method) mm.

Isolation	S. aureus			Klebsiella		
	100	75	50	100	75	50
Ni	11a	10 ab	8 bc	9 abc	8 bc	7 c
Cu	11a	10 ab	9 abc	8 b	7 c	6 d
Tukey's	0.458					

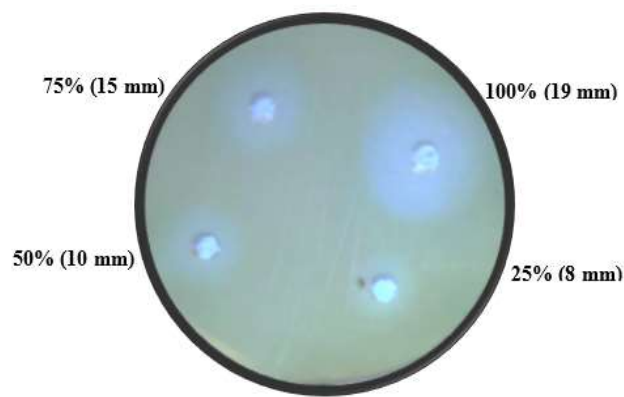


Figure: (2). Antibacterial activity of nickel against S. aeruginosa

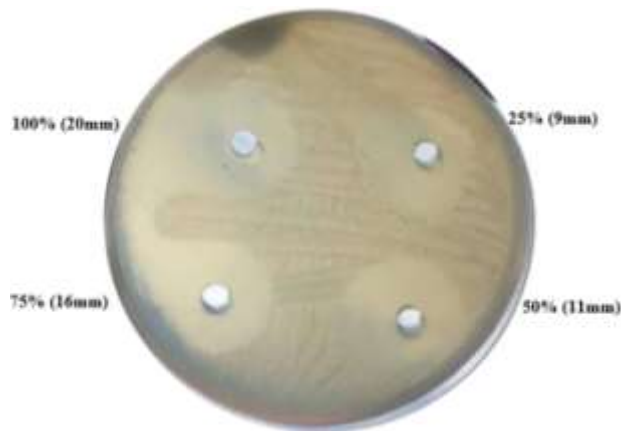


Figure: (3). Antibacterial activity of copper against S. aeruginosa

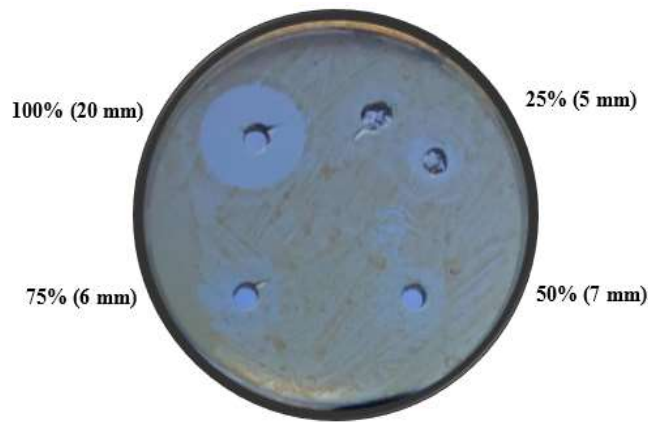


Figure: (4). Antibacterial activity of nickel against klebsiella.

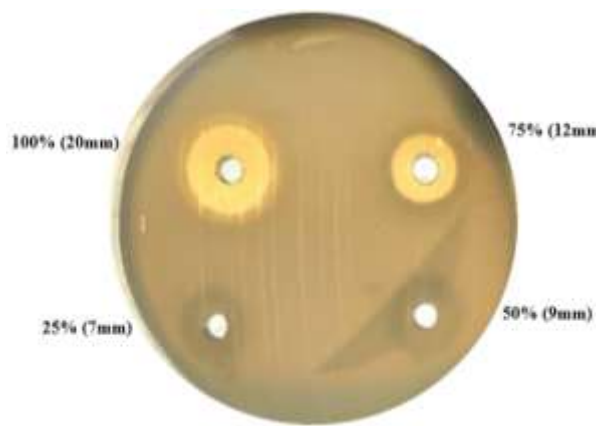


Figure: (5). Antibacterial activity of copper against kleb

### 3.4. Effect of different concentrations of metals on the growth of bacterial species

The effect of different concentrations (100%, 75%, and 50%) of metals solutions (Ni, Cu, and Fe) on isolated bacteria were illustrated in Table (5 - 7). The results showed that the antibacterial activity, no growth of bacteria at high concentration of (100%), whereas there is presence for bacteria growth at concentrations (50 & 75 %). In addition, showed bacteria growth for the species (Klebsiella and S.aureus).

Table: (5). Effect of Nickel concentrations on special bacteria.

Ni	100%	75%	50%
<b>Klebsiella</b>	-	+	+
<b>S. aureus</b>	-	+	+

\*Positive (+), and negative (-)

Table: (6). Effect of copper concentrations on selected bacteria.

Cu	100%	75%	50%
<b>Klebsiella</b>	-	+	+
<b>S. aureus</b>	-	+	+

Table: (7). Effect of iron concentrations on selected bacteria.

Fe	100%	75%	50%
<b>Klebsiella</b>	-	+	+
<b>S. aureus</b>	-	+	+

Figure: (6). Total carbohydrate and Total protein content (ppm) in the studied samples.

#### 4. Discussion:

Using specific protocols, the 112 samples which collected in this study were obtained for this investigation from a variety of sources at locations where contamination is more likely. Where containing *Pseudomonas* is the most common, it also aligns with the findings of (Rishi et al. 2013).

In Libya, a study which carried out at Tripoli Central Hospital, recorded presence of Various bacterial isolates from general surgery departments and medical laboratories, where about 35%, *Klebsiella* *Staphylococcus* (8.9%) and *Pseudomonas* (29%). Were recorded in this investigation on Tripoli Central Hospital (Giacometti et al., 2000).

As per the results of this investigation, the element nickel exhibited antibacterial activity at a concentration of 100, as demonstrated by its effective activity measured by spectrophotometry, MIC, and the agar diffusion methods. The discovery was made that the enhanced antibacterial activity that permeates the bacterial cell and reaches the membrane surface is due to the properties of both the metal ion and the ligand surrounding it. The microbial cell and its internal milieu. The impact of this flow on bacterial metabolism is the destruction of the organelles. The findings of this study were agreement with the results recorded by (Haghshenas and Faraji, 2016). Which they were examined the MIC and UV spectroscopy methods for assayed the antibacterial activity of some bacteria using electron microscopy on *E. coil* and *S. aurous*.

In addition, some studies recorded that the Nickel (Ni) solutions showed antibacterial activity on some species of bacteria (Otuokere et al., 2018). For both Gram-positive and Gram-negative bacteria did nickel complexes effectively combat both. In this study the results of copper concentrations (Cu) on the isolated bacteria were agreement with those results which shown by (Santos et al., 2012) which they reported that the different Copper (Cu) showed a good effect on inhibition of bacterial growth.

Some studies reveled that effect of Meal solutions on the anti-bacterial activity is due to their great capacity to pierce bacterial membranes; copper is thought to possess the greatest bactericidal potential. The capacity to attain a very high killing effectiveness for bacteria that are in close contact for a brief length of time is what makes metallic copper or copper surfaces unique. This is because of the way that copper atoms are structured, particularly how quickly they can give or receive electrons, which is what makes copper an electrical conductor. unbound electrons throughout Microbes' activity can be suppressed by interactions with microbial proteins during contact, (Santos et al., 2012).

Another study reported that the ROS were produced rapidly, which damages DNA and the respiratory system, ultimately resulting in cell membrane damage (Li et al., 2016).

It was reported that the cell disruption occurs when there is a leakage of cellular components due to damage to the cell membrane. Copper destroys MRSA through DNA damage corroborate (Abraham and Florentine, 2021; Abraham et al., 2021). Their study declared that copper exposure renders many

microorganisms nonviable. It is suggested that frequently touched surfaces in healthcare institutions be replaced with copper or copper pipes, either immediately or over a predetermined period. This will significantly reduce the spread of illness.

(Abdullah et al., 2016) stated that the dodder plant extracts showed direct effective on *S. aureus* at highest antibacterial activity at 100% concentration. The study conducted by demonstrated that the plant extract had a superior effect on *S. aureus* than amoxilin-containing drugs. Gram-positive bacteria responded well to it, but gram-negative bacteria were unaffected. (Sen, 2018), found that the dodder extracts were resistant to the *Staphylococcus aureus*, *E. coli*, *Klebsiella* and *Pseudomonas* species.

(Mishra and Chan, 2016) reported that the dodder plant has showed antibacterial efficacy against both Gram-positive Gram-negative bacteria of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, the same results were recorded in this study.

Our results also agreement with A study which carried out by (Sonmez et al., 2019) where the Dodder plant extract was applied by used agar disk diffusion methods. They were selected bacteria species of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were the four bacteria examined, the results showed that the dodder plant extracts had sensitive and moderate antimicrobial activity against each of them. Due to its potential for application as a novel antibacterial agent.

These phytochemicals were found in noticeable amounts in Dodder and other fruit sections, according to a phytochemical study (Eldesoky et al., 2019).

Dodder is also a very rich source of phytochemicals that have demonstrated strong antioxidant properties. The health advantages of these phytochemicals have been emphasized in earlier research, and quantitative.

In this study also the iron (Fe) exhibited anti-bacterial activity, it was reported that iron compounds, whether in plasma or in different types of plasma clots, slightly or greatly increase the growth of bacteria, just as in genuine infection instances. Transferrin's iron saturation is well known (Gillon Ward et al., 1985).

The antibacterial effects of the pollutants are neutralized when they are accessible to bacteria. Revealed that iron has the ability to neutralize bactericidal effects. Early growth above 60% was seen upon microscopic analysis of plasma clots that had fast expansion by 90%. According to this, bacteria can more easily absorb iron for fast development at higher transferrin saturation levels (Paul Bullen et al., 1974).

It was reported that the velocity and iron uptake in vivo are intimately related. *Pseudomonas* growth is induced by bacterial infection, which also generates bacteria with enhanced iron use. It is now known that iron metabolism and bacterial pathogenicity are related (Sokol and Woods, 1984).

## **5. Conclusion**

This study used crude dodder extract and certain element concentrations to demonstrate antibacterial efficacy against both Gram-positive and Gram-negative bacteria. This study demonstrates that nickel metal exhibits 100% concentration-dependent bactericidal and antibacterial action. Similarly, copper's capacity to penetrate bacterial membranes, which results in damage to DNA, cell membrane damage, and cell death, had an effective effect. Furthermore, the alcoholic dodder extract is thought to have no effect *Pseudomonas*, at high concentrations 100%, the extract has a greater impact on Gram-positive bacteria than on Gram-negative bacteria. All of the data used in this study of it, has different contents.of

phytochemical substances that, at varying, medium to low concentrations, contain phenols, alkaloids, amino acids, and minerals and have antioxidant, antibacterial, and anticancer effects.

**6. Recommendation:**

1. A hospital infection surveillance system must be introduced to reduce the rate of nosocomial infection among patients and to obtain better treatment options.
2. More investigation, study, and survey must be done among Libyan medical centers to identify pathogens cause hospital infection.
3. Health authority must consider the results and data of this study.
4. The plant extracts and mineral compounds used in this study as alternatives to antibiotics are less toxic, more effective, and less expensive than other metals such as silver. Furthermore, these extracts are free of adverse effects

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