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Original article

Using the extracts of the (Dodder) plant and the concentrations of some metals as inhibitors for growth, the (*Pseudomonas*) bacteria isolated from some hospital rooms in Derna and Al-Bayda

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Abstract
This study aimed to isolate and identify 112 samples from different departments in Derna and Al Bayda hospitals to determine the most common bacteria. Standard microbiological methods were used to identify the isolated bacteria from the study areas, such as spectrophotometry, minimum inhibitory concentration (MIC), and agar diffusion. Alcoholic and aqueous extracts of the wormwood plant were also used, in addition to the concentrations of some elements (copper, nickel, and iron) as inhibitors of the bacteria. Statistical analysis was performed using Tukey's analysis software, MlNlTAB version 2021. Some chemical analyses included phytochemical examination, antioxidant content, phenolic compounds, and metals. Some of the elements showed that the most common was Pseudomonas aeruginosa, accounting for 22.2% of the total. The effects of the wormwood plant extracts were studied, and their effectiveness was compared with the concentrations of the elements used in the study. The results showed that Dodder plant extracts had no effect on Gram-positive bacteria, and that the most effective and influential elements were nickel at a concentration of 100%, followed by copper, and the least effective was iron.
Keywords: Bacterial Isolation, Dodder Extracts, Antimicrobial, Hospital-Acquired Infections.

Introduction

Before being admitted to the hospital, patients in low who do not currently have healthcare resources get health-associated infections (HAIs). Reducing and eliminating nosocomial infections can help address hospital-acquired illnesses, which are serious problems that need to be carefully addressed globally. These infections do not affect one in particular. These might happen to everyone, patient or hospital staff, and raise the hospital's mortality rate [1]. Studies show urinary tract infections are among the most common causes of Hals (UTIs), infections of the circulatory system, respiratory tract infections (RTIs), and surgical site infections range from 18 to 101 [2]. As per a World Health Organization (WHO) assessment, 55 hos-HAIs were more common in 14 nations, accounting for 8.7% of hospitalized patients. This has led to an increase in both mortality and morbidity rates. Strong. The largest concentration of *P. aeruginosa* is 11% of all nosocomial infections, which have a high risk of morbidity and death. Non-fermenter Gram-negative organisms are the source of diseases, particularly in those with weakened immune systems. The kidney, urinary system, and upper respiratory tract are colonization sites. It is the source of UTIs, bacteremia, pneumonia, cystic fibrosis, and wound and surgical infections. Adhesions, hemolysins, exotoxins, proteases, and siderophores are a few significant virulence factors. A variety of processes opposing drugs are causing *P. aeruginosa* to become more resistant [3]. Dodders (Cuscuta spp.) are the most common group of parasitic weeds that are important to agriculture and the economy. Dodder cannot finish its life since it is an obligatory parasite in cycle. Lists numerous popular names for the annual plant called dodder, including angel's hair, golden thread, and strangle weed. Without leaves or roots, seedlings are made entirely of stem tissue. Typically, golden to orange in hue, stems are thin threads. Some species of dodder may have minimal amounts of chlorophyll, even though they depend on their host for nutrition to survive. Research has demonstrated that the components of C. campestris, or dodder, have the following effects: They are effective against atopic dermatitis [4]. They are also effective against some plant-pathogen fungi [5]. They have an anticancer effect [6]. They have analgesic, hypothermic, anti-inflammatory, and anti-proliferative effects [7]. Family: Cuscutaceae. Scientific Name: Cuscutaplaniflora Ten. Vernacular Name: Vern. Hariri Essayer.

The body of knowledge about how important metal ions are to biological systems is constantly expanding. Thus far, research has demonstrated that the majority of organisms need the manganese, iron, nickel, copper, and zinc first-row transition metals. Certain absorption processes are used by bacteria to obtain the necessary [8]. Nickel is thought to be connected in some manner to the structure or operation of proteins. It is found in nucleic acids, specifically RNA and DNA. It has focused mostly on the synthesis of nickel complexes with ligands due to their biological properties [9]. 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 [9]. 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

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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 [10]. 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 through the cell wall. Consequently, combination treatments provide a number of benefits, including a decreased risk of developing [11,12]. The aims of this study are to isolate and identification of pathogenic bacteria from some departments at Al-Bayda and Derna city hospital, then using some metal ion solutions and Dodder plant extracts to inhibit the bacterial species.

Methods

Study locations

This study was conducted at Al-Bayda Medical Center in Al-Bayda and Al-Wehda Hospital in Derna, Libya, (2024). Al-bayda and Derna are cities located in the northeastern region of Libya, as shown in (Figure 1).

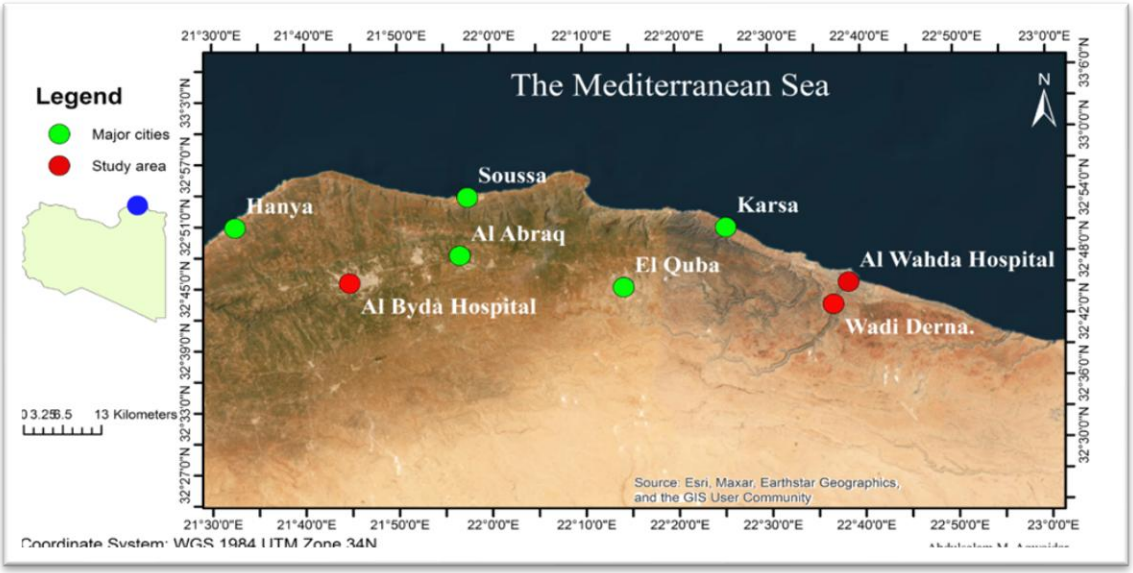

Figure 1. The area of study (Derna and Al-Bayda City Hospital), Libya

Isolation and identification

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 the 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 the women's Department only, which consisted of cauterization devices, monitoring devices, emergency carts, sterilization devices, and seats.

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 [13].

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 [13].

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Antimicrobial investigation
Spectrophotometric method
The spectrophotometric method, which was carried out in this study was involved measuring the mixture of isolated bacteria with the suggested metal ion solutions. A suspension of 1.5×108 CFUL mL 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, 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. 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 [14].

Agar well propagation 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 McFarland 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 [15].

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 [16]. In this study, each one of the concentrations 25, 50, 75, and ppm was added to the Nutrient Agar (NA) medium at 50°C. The suspension was distributed on plates and then left for a few minutes to dry. The plants 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. The results were recorded as the presence or absence of growth.

Preparation of extracts
Solvent extraction
The powdered materials of the plant Cuscuta dodder were extracted with different solvents (water and ethanol), where 10 grams of plant powder were combined with 100 milliliters of both aqueous and non-aqueous solvents (ethanol). At 60°C, crude extract was evaporated, and with the rotary evaporator, the extracts were collected and stored at 4°C until further use [17-25].

Phytochemical screening
All the phytochemical screening tests were carried out according to standard methods. The methods are described as follows [26–40]:

Test for sterols and/or triterpines
Libermann-Burchard's test
A small amount of concentrated sulfuric acid was put down the side of the dry test tube after 0.3 ml of acetic anhydride and 1 ml of the chloroform extract of each sample had been added. Chloroform solutions turn green when sterols and/or triterpenes are present, whereas reddish-violet coloration is formed at the intersection of the two layers.

Test for flavonoids
The extracts (alcohol and aqueous) of the tested herbal plants were further extracted with 1% hydrochloric acid; each extract was subjected to the following test: 10 ml of each extract is rendered alkaline, where a faint yellow color is produced in the presence of flavonoids

Test for alkaloids
The extracts of the tested herbal plants were further extracted with 20 ml of dilute hydrochloric acid, cooled, and rendered alkaline with a dilute ammonium hydroxide solution, and then extracted with chloroform.

Dragendorff the preparation of the reagent
Solution (a): 10 milliliters of acetic acid and 40 milliliters of distilled water were combined to dissolve approximately 0.85 grams of basic bismuth nitrate. Solution (b): 20 milliliters of water were used to dissolve around 8 grams of potassium iodide. Standard response: Solutions (a) and (b) are combined in

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equal volumes. On filter paper, a few drops of chloroform extract were placed, allowed to dry, and then sprayed with the reagent. When alkaloids are present, an orange hue is seen.

Determination of metal concentrations

The concentrations of the studied metals of Nickel, Copper, and Iron were determined by atomic absorption, where 0.5 g of leaves of the Dodder plant was digestion with 5 ml nitric acid (Concentrated), in the presence of 25 ml of distilled water. The mixture was heated until dry, then filtered, and the volume was completed with distilled water to 100 ml. The concentrations of the studied metals were expressed as (ppm). This method was described to estimate the heavy metals in soil samples as leafs, stems, water, soil, and others [49-80].

Statistical Analysis

Analyzed the normality distribution according to Shapiro, wilk Ryan Joiner and klomogorovsmirnov at a significance level of 0.01 A factorial experimental with a Completely Randomized Design (CRD) It was the first experience three factors for the study factor one the isolates (Klebsiella pseudomonas and S.aureus) factor two the elements (Fe.Cu and Ni) and Dodder plant and factor three the concentrations at the levels (25, 50, 75, and 100 %) The optical density (OD) was measured by the turbidity measurement method. As for the second experiment, using agar well diffusion techniques, the first factor was the isolates (*Klebsiella pseudomonas* and *S.aurous*), the second factor was the elements (Fe, Ni and Cu) Dodder plant, and the third factor was the concentrations (5, 10, 25, 50, 75, and 100 %) The comparison between the means was analyzed using Turkey's test statistical analysis was done using the MlNlTAB program by version 2021.

Results

Isolation and identification of bacterial species

Colonies appeared on transitional media and were confirmed by biochemical tests and Gram stain, where, under the microscope, A rod-shaped colony appeared, motile, gray in color, with a distinctive odor, requiring an oxidase test. A drop of the colony appeared on a filter paper, and a drop of oxidase solution was placed on it. It appeared purple, *Pseudomonas*, distinguished by its green color on NA medium, anaerobic. The results of isolation bacteria from the isolated samples, which were used in this study, showed that the species of bacteria, including 22% Pseudomonas, is the most common (Table 1).

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

Isolation bacteria	Average
pseudomonas	22.261a±16.49
Tukey's	0.2652 ***

For the Spectrophotometer method the Effect of metal concentrations and Dodder plant extract on isolated bacteria inhibition by spectrophotometric method recorded that: The high spectrometer was set to measure the transmittance 600nm which refer to the optical density at a wavelength of 600 nm 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%) and dodder extract 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 in Tables (2).

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

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On the other side the effect of concentrations (50, 75 and 100 %) of Dodder plant extract showed antibacterial activity values of (17.80, 5.93, and 6.41 nm), (12.36, 6.36, and 6.40 nm), and (10.26, 4.33 and 15.1 nm) on *Pseudomonas*, the results were illustrated in Table 3.

Table 2: Effect of metal concentrations and plant extracts on 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 and the extraction of the dodder plant, 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 ś test using the MlNlTAB program by version 2021, Among the metal concentrations and the extract of the dodder plant, the one with the highest permeability was nickel at a concentration of 100% and copper at a concentration of 100%, then iron and the extract of the dodder plant were the least 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 (OD.. nm).

Isolation	***Pseudomonas***		
Concentrations	**100%**	**75%**	**50%**
Dodder	6.4l	6.4	15.1
Ni	95.767 a	14.076	10.2
Fe	45.733 c	2.9	5.3
Cu	44.767 c	11.067	9.433
Tukey's	0.9185***		

For the Agar diffusion method, the Effect of metal concentrations and Dodder plant extract on isolated bacteria inhibition by Agar diffusion method was recorded.

The effect of the plant extract concentrations of 50, 75, and 100% did not give antibacterial activity on the isolated bacteria (Table 4) and (Figures 2 & 3). On the other side, the solutions of metals (Fe, Ni, and Cu) for concentrations of (50, 75, and 100 %) showed small values of antibacterial activities compared with the values of values obtained by spectrophotometric methods. In addition, the concentrations of (Fe) did not show any antibacterial activities. The concentrations (100 %) of Nickel solutions showed values of (11, 9, and 11 mm), while the isolated bacteria, Pseudomonas, whereas the at concentrations (75 %) gave inhibition values. In addition, for the concentrations of (10, 8, and 10 mm) and the Copper concentrations of (50 %) gave inhibition values of (8, 7, and 8 mm), and the concentration of copper solution of (50 %) showed (9, 6, and 1.33 mm) against *Pseudomonas* (Table 4).

Table 4. Effect of antibacterial activity of different metal concentrations, and isolation by the agar diffusion method) mm.

Isolation	***Pseudomonas***		
Conc.	100	75	50
Dodder	of	of	of
Ni	11 a	10 ab	8 bc
Cu	8 bc	6 d	1.33 e
Tukey's	0.458		

Effect of different concentrations of metals on the growth of bacteria

The effect of different concentrations (100%, 75%, and 50%) of metal solutions (Ni, Cu, and Fe) and plant extracts of Dodder on isolated bacteria was illustrated in (Table 5). The results showed that the antibacterial activity, no growth of bacteria at high concentration of (100%), whereas there are presence for bacteria growth at concentrations 50 & 75. In addition, the Dodder plant extract showed bacteria growth for the *Pseudomonas* (Table 5).

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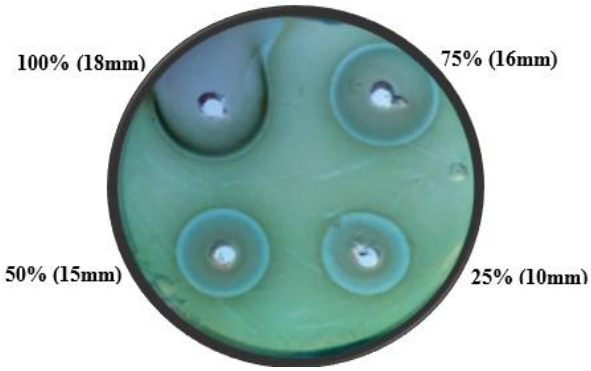

Figure 2: Antibacterial activity of nickel against P. aeruginosa

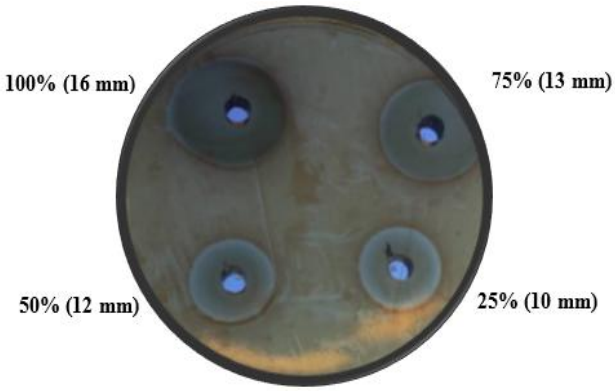

Figure 3. Antibacterial activity of copper against P. aeruginosa

Table 5. Effects of metals and Dodder extracts of concentrations on bacteria.

P. areginosa	**100%**	**75%**	**50%**
Ni	-	+	+
Cu	-	+	+
Fe	-	+	+
Dodder	+	+	+

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

General phytochemical screening of all parts of the Dodder

(Tables 6 and 7) showed the general phytochemical screening of the Dodder extract. The results recorded the presence of cardiac glycosides, tannins, flavonoids, sterols, and/or triterpenes, alkaloids, and carbohydrates and/or glycosides, and absence of anthraquinone and saponins in both leaves and stems in alcohol extract. On the other side, for the water extract, the results of phytochemical screening showed the presence of all constituents, and the absence of Flavonoids in both leaves and stems of *Dodder* plant extract.

Dodder has compounds similar to antioxidants that may help inhibit the growth of cancer cells Tables 6 and 7. Furthermore, phenolic substances have been employed as purgatives, including anthraquinones

Table 6: Phytochemical screening of water extracts.

Phytochemical screening test	Leaves	Stems
Sterols and/or triterpenes	+++	+++
Flavonoids	-	-
Anthraquinone	+++	+++
Tannins	+++	+++
Alkaloids	+++	+++
Saponins	+++	+++

***+:** low amount, **++:** moderate amount, **+++:** high amount, and-**:** absent*

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Table 7. Phytochemical Screening of ethanol extracts.

Phytochemical screening test	Leaves	Stems
Sterols and/or triterpenes	++	++
Flavonoids	++	+
Anthraquinone	-	-
Tannins	++	++
Alkaloids	++	++
Saponins	-	-

***+:** Low Amount, **++:** Moderate Amount, **+++:** High Amount, And**-:** Absent*

Contents of Minerals and metals of the Dodder extract

The results of the minerals and metal contents of the studied plant (Leaves of Dodder) are given in (Figures 4 & 5). High content (82 ppm) of minerals was obtained for sodium (Na), whereas the potassium (K) and calcium contents were 16 and 14 ppm, respectively. Also, the contents of (Iron (Fe), Copper (Cu), and Nickel (Ni)) were (0.6, 0.42, and 0.38 ppm), respectively.

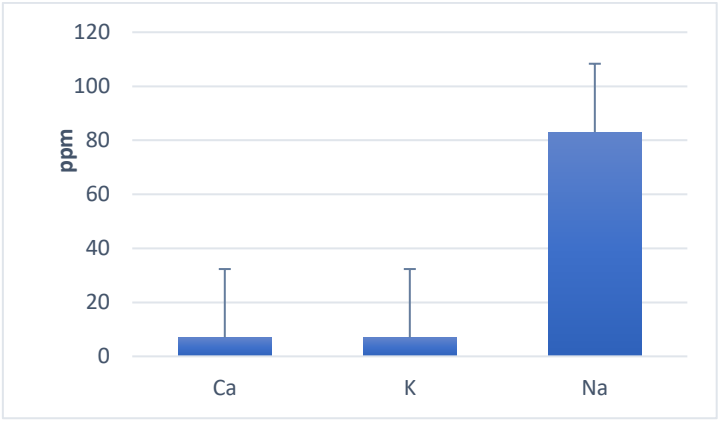

Figure 4: Minerals (Ca, K, and Na) and content (ppm) in extracts Dodder plant studied.

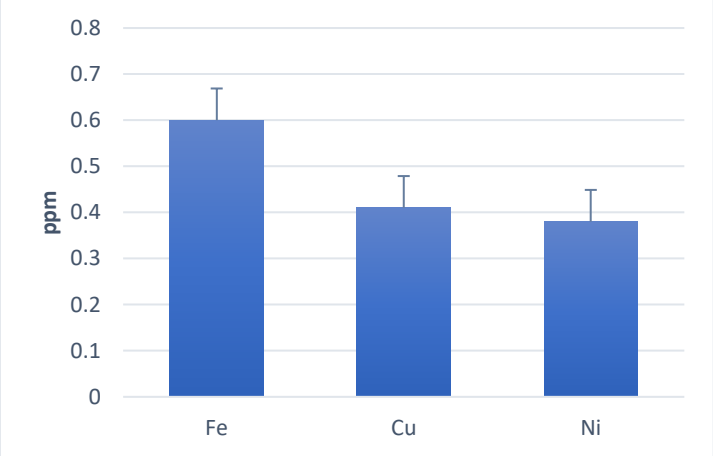

Figure 5: Minerals (Fe, Cu, and Ni) and content (ppm) in extracts Dodder plant studied.

Total phenol and antioxidant contents

(Figure 6) shows the concentrations of total carbohydrate and Total protein content (ppm) in the studied plant. There is a significant content of anti-oxidant and total phenol compounds of values (1.22 and 0.90 ppm), respectively. The recorded concentrations of carbohydrate and protein contents were (1.32 and 0.18 ppm), respectively.

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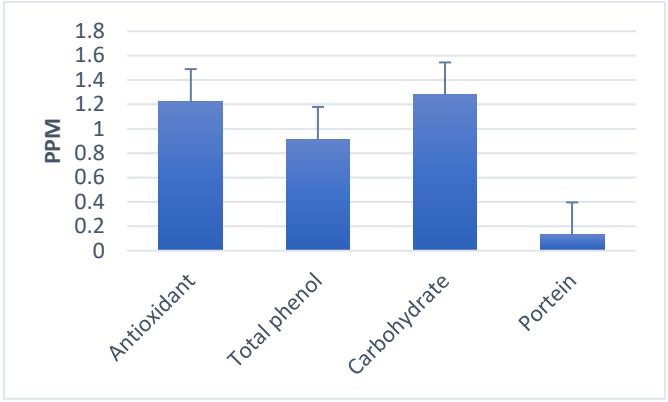

Figure 6: Total carbohydrate and Total protein content (ppm) in the studied samples.

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 *Pseudomonas* is the most common, it also aligns with the findings of a study that reported the same results [81].

It was stated that the dodder plant extracts showed direct effect on S. aureus at the highest antibacterial activity at 100% concentration [82]. The study conducted demonstrated that the plant extract had a superior effect on *S. aureus* than amoxicillin-containing drugs. Gram-positive bacteria responded well to it, but gram-negative bacteria were unaffected. It was found that the dodder extracts were resistant to the Staphylococcus aureus, *E. coli*, *Klebsiella,* and *Pseudomonas* species [83]. It was concluded that reported that the dodder plant has shown antibacterial efficacy against both Gram-positive and Gram-negative bacteria of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis,* and *Pseudomonas aeruginosa* [84]; the same results were recorded in this study.

Our results also agree with A study carried out by some studies [85], where the Dodder plant extract was applied using agar disk diffusion methods. The 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 [86]. *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 quantitatively.

It was reported that cell disruption occurs when there is a leakage of cellular components due to damage to the cell membrane. Copper destroys MRSA through DNA damage, corroborating [87]. 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.

In this study, the iron (Fe) also exhibited anti-bacterial activity. It was reported that iron compounds, whether in plasma or 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 [88]. The antibacterial effects of the pollutants are neutralized when they are accessible to bacteria. Revealed that iron can neutralize the 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. 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.

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 not to affect 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 have 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.

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Conflict of interest. Nil

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