

The Effect of Methanolic Extract of *Quercus infectoria* Against Some Causative Agents of Diarrhea and Eliminate their Antimicrobial Resistance

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Abstract

We obtained 124 bacterial isolates including: *Escherichia coli*. I (35 isolates 7%) isolates, *E. coli* II (8 isolates 1.6%), *E. coli* III (17 isolates 3.4%), *E. coli* IV (22 isolates 4.4%), *Shigella dysenteriae* (8 isolates 1.6%), *Salmonella arizonae* (16 isolates 3.2%), *Salmonella typhi* (12 isolates 2.4%) and *Vibrio cholera* (6 isolates 1.2%) Sensitivity test showed high resistance to amoxicillin/clavulanic acid, amoxicillin, ampicillin, erythromycin, streptomycin, nitrofurantoin, cefixime, rifampicin, tetracycline, trimethoprim - sulfamethoxazole and tobramycin. Most of the isolates appeared sensitive to nalidixic acid, gentamycin, amikacin, doxycyclin, cefotaxime, cephalexine and chloramphenicol. All isolates were sensitive to ciprofloxacin. The MIC of the methanol extracts of *Quercus infectoria* against different pathogenic bacteria was ranging between 2.5 mg/ml - 20.0 mg / ml and the MBC was 1.25 mg/ml - 20.0mg/ ml. The isolates revealed high resistance to most widely used antibiotic, the methanol extracts of *Quercus infectoria* showed high potential as antibacterial agent.

Key words: Antimicrobial activity, *Quercus infectoria*, pathogenic bacteria.

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تأثير المستخلص الميثانولي لنبات *Quercus infectoria* على بعض مسببات الاسهال والقضاء على مقاومتها للمضادات الحيوية

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الخلاصة

تم الحصول على 124 عزلة تضمنت 35 (*E. coli*. I (% 7) و *E. coli* II و 8 (1.6%) عزلات و 17 (3.4%) عزلة *E. coli* III و 22 (4.4%) عزلة تعود الى *E. coli* IV و 8 (1.6%) عزلة تعود الى *Shigella dysenteriae*، 16 (3.2%) عزلة تعود الى *Salmonella arizonae* 12 و (2.4%) عزلة، *Salmonella typhi* و *Vibrio cholera* 6 (1.2%) عزلات. وأظهر اختبار الحساسية مقاومة عالية لأموكسيسيلين / حمض كلافولانيك، أموكسيسيلين، الأمبيسلين، الارينثروميسين، الستربتومايسين، نيتروفورانتوين، سيفيكسيم، ريفامبيسين، التتراسيكلين، تريميثوبريم - سلفاميثوكسازول و توبراميسين. ومعظم العزلات اظهرت حساسية لحمض الناليديكسيك، جنتاميسين، أميكاسين، دوكسي سيكلين، سيفوتاكسيم، سيفاليكسين والكلورامفينيكول. وكانت جميع عزلات البكتيريا قيد الدراسة حساسة للسيبروفلوكساسين. كان قيمة التركيز المثبط الأدنى للمستخلص الميثانولي لنبات *Quercus infectoria* ضد البكتيريا المسببة للإسهال مختلفة تتراوح بين 2.5 ملغ / مل - 20.0 ملغ / مل و قيمة التركيز المثبط الأدنى كان 1.25 ملغ / مل - 20.0 ملغ / مل. وأظهرت العزلات مقاومة عالية للمضادات الحيوية الأكثر استخداما على نطاق واسع، وأظهر المستخلص الميثانولي لنبات *Quercus infectoria* فعالية عالية مضادة للبكتيريا.

الكلمات المفتاحية: الفعالية المضادة للمكروبات، *Quercus infectoria*، البكتيريا المرضية.

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Introduction

The accelerated emergence of antibiotic resistance among the prevalent pathogens is the most serious threat to the management of infectious diseases [1]. The evolution and spread of various mechanisms of antimicrobial resistance among common human pathogenic members of Enterobacteriaceae is of increasing concern and led the research for more novel antimicrobial compounds [2]. Many local plants are cheap, readily available and widely used in traditional folk medicine since they produce a diverse range of bioactive compounds. Unlike human-synthesized medicine which normally consists of only a single bioactive compound, the plant extracts may contain more than one bioactive ingredients which synergistically work against a particular disease. In addition, being from nature is normally perceived as safer and therefore, more acceptable by humans [3]. *Quercus infectoria* Olivier (Fagaceae) is a small shrub mainly present in Greece, Asia Minor, Iran, Iraq, Turkey and Syria. It is locally cultivated for its valuable medicinal properties. The medicinal properties of the plant have been a subject of numerous investigations. In traditional folk medicine, the galls are extracted with hot water for use as a gargle to relieve inflamed tonsils or directly applied onto the inflamed skin to reduce swelling. In addition to using *Q. infectoria* extracts as essential oil in food preparations, nutraceuticals or cosmetic anti-aging, they have also been known to produce many bioactive compounds [3, 4] with antibacterial [5], antifungal [6], antidiabetic [7], local anaesthetic [8], antiviral [9], and anti-inflammatory [10] activities. The active compounds in *Q. infectoria* were tannic acid, Glycosides such as cortis and tannins. Hence, this study was designed to assess the effectiveness of different solvent extracts of *Q. infectoria* on the growth of some gram-negative bacteria [11].

Materials and Methods

Sample collection

Five hundred stool samples were collected from infants and children (below ten years) suffering from diarrhea and admitted to the Pediatric and Maternity Hospital in Erbil City.

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Identification of bacteria

Identification of bacteria was done on the basis of morphological, cultural characteristics by using a selective medium, then with biochemical tests and further by using an API20E system [5].

Preparation of crude extracts

Q. infectoria was used in the present study, classified by Dr. Abdul-Hussein Al- Khayat, College of Education, Salah Al- Deen University. The collected medicinal plants were washed with distilled water and then dried for the preparation of methanol extracts [12].

Antibiotic sensitivity test

Antibiotic susceptibility test of all bacteria isolates was determined by the standard Kirby-Bauer disk diffusion method [13]. These antibiotics used were Chloramphenicol, Ampicillin, Amoxicillin/clavulanic acid, Amoxicillin, Tetracycline, Streptomycin, Trimethoprim-sulfamethoxazole, Erythromycin, Cephalexine, Rifampicin, Cefixime, Cefotaxime, Doxycyclin, Gentamycin, Nitrofurantoin, Nalidixic acid, Tobramycin, Ciprofloxacin and Amikacin (Bioanalyse). Bacterial cultures suspension equivalent to 0.5 tube McFarland turbidity standards were spread on Mueller-Hinton agar plates and incubated at 37°C for 24 hours, and then the diameters of the inhibition zones were measured. Results were expressed according to the criteria recommended by the CLSI [14].

Determination of MIC and MBC values

To study the effect of different antimicrobials on all isolates of bacteria, Mueller-Hinton agar was used as a growth medium. After sterilization and cooling at 45°C, the final concentration of the selected antibiotics was added to the media and poured into sterile Petri dishes. After solidification, the plates were inoculated by streaking method with bacterial isolates then incubated at 37°C for 24 hours. The results were recorded next day [15].

The antibacterial activity of the extracts was determined by evaluating the MIC using the Micro Broth dilution method, serial dilutions of the extract were prepared (0.0195 mg/ml to

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20 mg/ml) directly into nutrient broth in micro plates. The wells were seeded with culture at final inoculums of $1E6$ bacteria/ ml. The experiment was performed in triplicate. The bacterial suspensions were used as positive control and extracts in broth were used as negative controls. Then the plate was covered with a sterile plate sealer. The contents of each plate were mixed on plate shaker at 300 rpm for 20 seconds and then incubated at 37°C for 24 hours. Microbial growth was determined at 600nm using the ELX800 universal micro plate reader. The results were recorded in the form of MIC defined as the minimum amount of the extract that inhibits the growth of the microorganisms. While the least concentration showing no visible growth on agar subculture was considered as MBC value [9].

The inhibitory effect of different concentrations of plant extraction

The extracts of *Q. infectoria* were dissolved in sterile distilled water to a final concentration of 50mg/ml. The disc diffusion method was used to evaluate the antibacterial activity. Sterile filter paper discs were impregnated with 100 μl of each of the extracts, placed on Mueller Hinton agar plate incubated for 24 h. at 37°C . Distilled water served as negative control and ciprofloxacin was used as standard antibiotic to confirm that all the microorganisms tested were inhibited by the antibiotic. The antibacterial activity was evaluated by measuring the diameter of the inhibition against the tested isolate [16].

Results

In this study 124 isolates have been used and these included: *E. coli* I 35 (7%) isolates, *E. coli* II 8 (1.6%) isolates, *E. coli* III 17 (3.4%) isolates, *E. coli* IV 22 (4.4%) isolates, *Shigella dysenteriae* 8 (1.6%) isolates, *Salmonella arizonae* 16 (3.2%) isolates, *Salmonella typhi* 12 (2.4%) isolates and *Vibrio cholera* 6 (1.2%) isolates. The susceptibilities of all the isolates were tested against 19 antimicrobials. The *E. coli* I showed resistance to chloramphenicol (95%), ampicillin (90%), amoxicillin/clavulanic acid (86%), amoxicillin (80%), tetracycline (60%), streptomycin (56%), trimethoprim- sulfamethoxazole (50%), erythromycin (44%), cephalaxine (36%), rifampicin (32%), cefixime (30%), cefotaxime (30%), doxycyclin (26%), gentamycin (20%), nitrofurantoin (9%), nalidixic acid (9%) and tobramycin (8%). All isolates were sensitive to ciprofloxacin and amikacin.

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The *E. coli*. II showed resistance to amoxicillin (92%), chloramphenicol (90%), ampicillin (87%), amoxicillin/clavulanic acid (85%), tetracycline (65%), streptomycin (50%), trimethoprim-sulfamethoxazole (45%), erythromycin (40%), cefixime (39%), cephalaxine (39%), rifampicin (30%), cefotaxime (30%), doxycycline (25%), gentamycin (18%), tobramycin (10%), nalidixic acid (9.5%) and nitrofurantoin (8%). All isolates were sensitive to ciprofloxacin and amikacin. The *E. coli*. III showed resistance to amoxicillin/ clavulanic acid (90%), ampicillin (89%) amoxicillin (88%), chloramphenicol (80%), tetracycline (70%), streptomycin (52%), trimethoprim-sulfamethoxazole (45%), erythromycin (42%), cefixime (35%), cefotaxime (35%), cephalaxine (35%), rifampicin (29%), doxycyclin (28%), gentamycin (18%), nalidixic acid (10%), tobramycin (8.5%) and nitrofurantoin (8.5%). All isolates were sensitive to ciprofloxacin and amikacin. The *E. coli*. IV showed resistance to amoxicillin / clavulanic acid (89%), ampicillin (89%), chloramphenicol (85%), amoxicillin (81%), tetracycline (66%), streptomycin (55%), trimethoprim-sulfamethoxazole (55%), erythromycin (43%), cefixime (34%), cefotaxime (33%), rifampicin (30%), cephalaxine (30%), doxycyclin (22%), gentamycin (22%), nitrofurantoin (9.8%), nalidixic acid (9.5%) and tobramycin (8%). All isolates were sensitive to ciprofloxacin and amikacin (Figure 1).

Salmonella arizonae showed resistance to streptomycin, nalidixic acid, erythromycin and cephalaxine (100% each), amoxicillin (90%), amoxicillin / clavulanic acid (80%), tetracycline (80%), gentamycin (80%), ampicillin (70%), cefixime (70%), tobramycin (70%), rifampicin (50%), trimethoprim-sulfamethoxazole (30%), amikacin (20%), cefotaxime (20%). All isolates were sensitive to ciprofloxacin, nitrofurantoin, doxycyclin, and chloramphenicol.

Salmonella typhi showed resistance to amoxicillin, erythromycin, cephalaxine and nalidixic acid (100% each), tobramycin (80%), chloramphenicol (80%), ampicillin (80%), streptomycin (80%), amoxicillin / clavulanic acid (75%), rifampicin (70%), tetracycline (70%), trimethoprim-sulfamethoxazole (70%), gentamycin, (60%), cefixime (30%), amikacin (25%), cefotaxime (20%) and doxycyclin (5%). All isolates these were sensitive to ciprofloxacin and nitrofurantoin.

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Shigella dysenteriae showed resistance to amoxicillin / clavulanic acid, amoxicillin, ampicillin, and streptomycin (100% each), amikacin (90%), tetracycline (80%), gentamycin (80 %), tobramycin (70 %), cefixime (50 %), erythromycin (50 %), rifampicin (50 %) and trimethoprim-sulfamethoxazole (30 %). All isolates were sensitive to ciprofloxacin, nalidixic acid, cefotaxime, cephalaxine, doxycyclin, nitrofurantoin and chloramphenicol.

V. cholerae showed resistance to amoxicillin / clavulanic acid, amoxicillin, ampicillin, rifampicin, cephalaxine, and tobramycin (100% each), tri-methoprim-sulfamethoxazole (86%), erythromycin (50%), cefixime (40%), streptomycin (30%) and tetracycline (5%). All isolates were sensitive to ciprofloxacin, nalidixic acid, amikacin, gentamycin, cefotaxime, doxycyclin, nitrofurantoin and chloramphenicol (Figure 2).

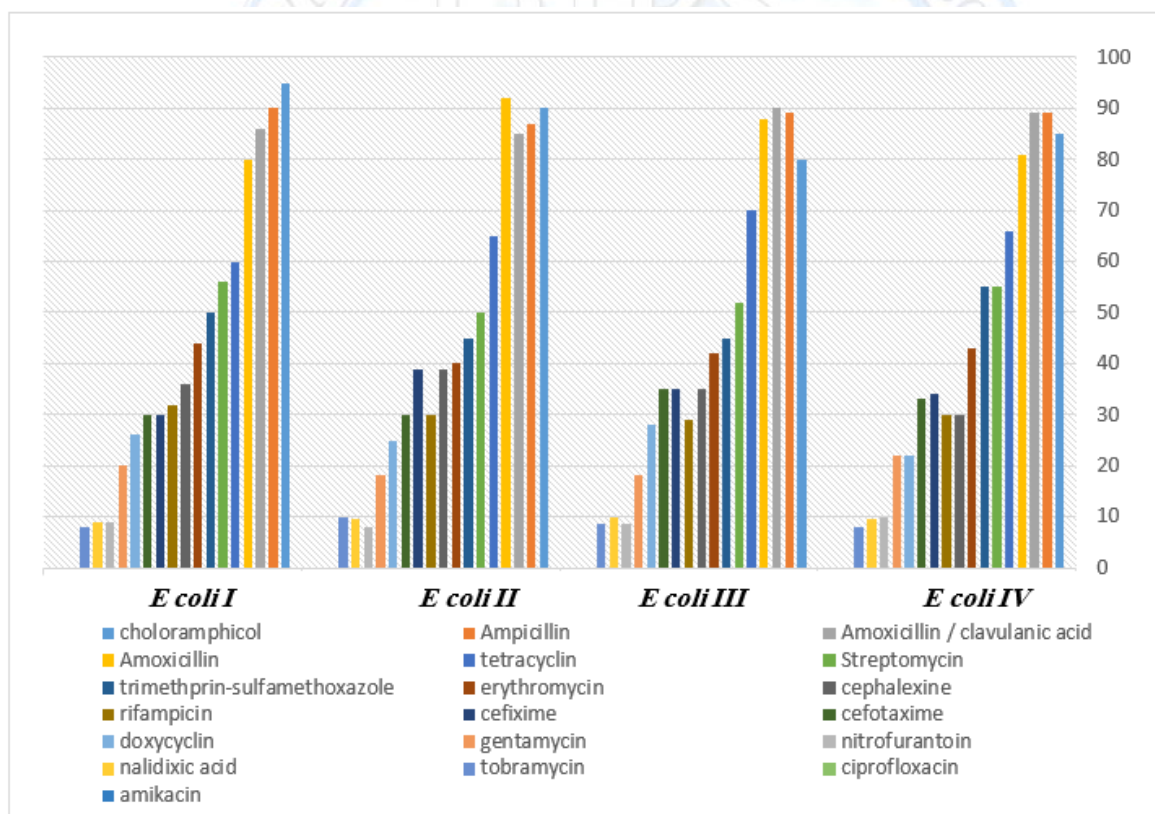


Figure 1: Susceptibility pattern (%) of *E coli I*, *E coli II*, *E coli III* and *E coli IV* in standard antibiogram.

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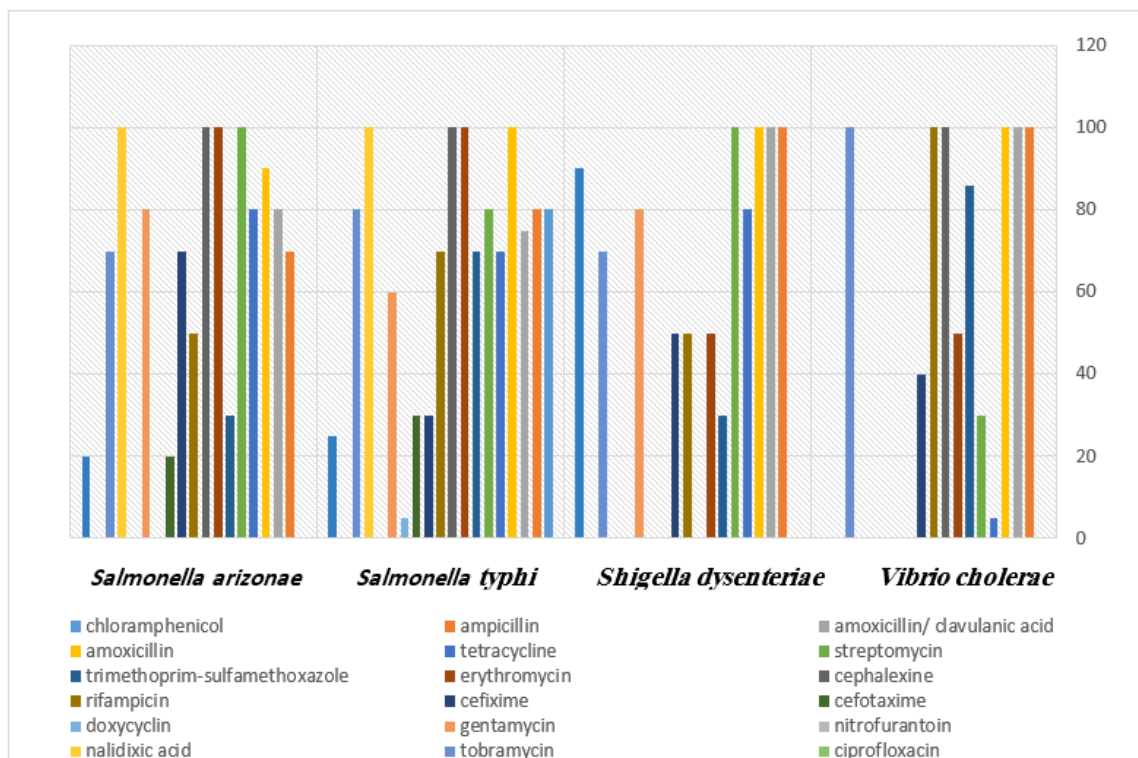


Figure 2: Susceptibility pattern (%) of *Salmonella arizonae*, *Salmonella typhi*, *Shigella dysenteriae* and *Vibrio cholera* in standard antibiogram.

Antibacterial activity of plant extracts against isolates showed that crude methanolic extract of *Q. infectoria* had no effect against all bacteria under study in a concentration of 0.625 mg/ml and less of then. But more active against *E. coli* polyvalent I and IV as evidenced by the size of the inhibition zones which was 30mm and *E. coli* II, III, *Sh. desenterae* and *V. cholera* of 15mm each, *S. arizona* and *S. typhi* 10 mm each in a concentration of 20.0 mg/ml. The result of antibacterial activity of methanolic extract of *Q. infectoria* against *E. coli* I, II, III, IV, *Sh. desenterae*, *S. arizonae*, *S. typhi* and *V. cholera* revealed that the diameters of the inhibition zones were 21, 12, 12, 25, 14, 6, 7 and 13 mm respectively in a concentration of 10.0 mg/ml. The results of antibacterial activity of methanolic extract of *Q. infectoria* against *E. coli* I, II, III, IV and *Sh. desenterae*, *S. arizonae*, *S. typhi* and *V. cholera* showed that the size of the inhibition zones were 15, 1, 1, 25, 11, 5, 5, and 10 mm respectively in concentration of 50 mg/ml. There were no effects of this extraction against *S. arizonae* and *S. typhi* at a

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concentration in 2.5 mg/ml, there only one effect on *E. coli IV* 10mm in concentration 1.25 mg/ml. All inhibition zones were compared with ciprofloxacin as a control, where the sensitive strains displayed an inhibition zone of 14-15 mm. Table (1).

Table 1: Antibacterial activity of methanol extract of *Q. infectoria* at different concentration against bacteria

<i>Vibrio cholerae</i>	<i>Salmonella typhi</i>	<i>Salmonella Arizona</i>	<i>Shigella desenterae</i>	<i>E. coli</i> polyvalent IV	<i>E. coli</i> polyvalent III	<i>E. coli</i> polyvalent II	<i>E. coli</i> polyvalent I	Concentration (mg/ml)	No.
15	10	10	15	30	15	15	30	20.0	1
13	7	6	14	25	12	12	21	10.0	2
10	5	5	11	25	1	1	15	5.0	3
6	0	0	7	15	8	5	6	2.5	4
0	0	0	0	10	0	0	0	1.25	5
0	0	0	0	0	0	0	0	0.625	6
0	0	0	0	0	0	0	0	0.1563	7
0	0	0	0	0	0	0	0	0.0781	8
0	0	0	0	0	0	0	0	0.0391	9
0	0	0	0	0	0	0	0	0.0195	10
15	14	14	15	15	15	15	15	Ciprofloxacin	C

C: control antibiotic, Inhibition zone (in mm)

The MIC of the methanol extract of *Q. infectoria* infection against different bacteria ranging between 2.5 mg/ml - 20.0 mg / ml and the MBC was 1.25 mg/ml - 20.0mg/ ml (Table 2).

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Table 2: Determination of MIC and MBC values of methanol extract of *Q. infectoria* against different bacteria

<i>Vibrio cholerae</i>	<i>Salmonella typhi</i>	<i>Salmonella Arizona</i>	<i>Shigella desenterae</i>	<i>E. coli</i> polyvalent IV	<i>E. coli</i> polyvalent III	<i>E. coli</i> polyvalent II	<i>E. coli</i> polyvalent I	Concentration (mg/ml)	No.
0.001	0.009	0.020	0.015	0.010	0.015	0.010	0.011	20.0	1
0.002	0.009**	0.015	0.020	0.011	0.020**	0.023	0.022	10.0	2
0.011**	0.010*	0.016**	0.021**	0.015	0.022	0.024**	0.022**	5.0	3
0.021*	0.140	0.018*	0.030*	0.019**	0.024*	0.020*	0.031*	2.5	4
0.126	0.240	0.130	0.040	0.020*	0.120	0.122	0.091	1.25	5
0.222	0.266	0.198	0.122	0.122	0.190	0.222	0.144	0.625	6
0.381	0.340	0.251	0.198	0.255	0.250	0.311	0.230	0.1563	7
0.455	0.397	0.311	0.265	0.299	0.311	0.345	0.298	0.0781	8
0.491	0.454	0.390	0.311	0.331	0.389	0.398	0.340	0.0391	9
0.511	0.511	0.452	0.390	0.411	0.450	0.444	0.388	0.0195	10
0.598	0.602	0.564	0.539	0.577	0.544	0.566	0.554	Control	11

*MIC, **MBC

Discussion

In this study, 124 bacterial pathogens were found in 24.8% of the total number of samples, of those 35 (7%) were identified as *E. coli*. I, 8 (1.6%) were *E. coli* II, 17 (3.4%) *E. coli* III, 22 (4.4%) *E. coli* IV, 8 (1.6%) *Shigella dysenteriae*, 16 (3.2%) *Salmonella arizonae*, 12 (2.4%) *Salmonella typhi* and 6 (1.2%) were identified as *Vibrio cholera*. These results were in agreement with the results of Brad *et al.* [6] and Behiry *et al.* [5] who found that *E. coli* was the most common isolate group and the percentage of infection with both *E. coli* and *Salmonella spp.* was 1.81% for each.

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Most isolates of *E. coli* were highly resistant to amoxicillin (80-90%), amoxicillin / clavulanic acid (85-90%) and ampicillin (81-92%). It was found that all isolates of *E. coli* were sensitive to cephalexine and ciprofloxacin and this finding is similar to what was obtained by Estrada-Garcia *et al.* [17] who found all isolates were susceptible to ciprofloxacin and cefotaxime. Ciprofloxacin and other quinolones are not approved for children because of the risk of damage to immature joints and most parental third generation cephalosporins (cefotaxime) are administered only in a hospital setting. *Shigella spp.* seems to be resistance to ampicillin, amoxicillin and rifampicine and these results are similar to those of Al-Shuwalli [18]. In addition *Shigella spp.* were found to be highly sensitive to ciprofloxacin, nalidixic acid and cefotaxime [19]. All *Salmonella spp.* were sensitive to aminoglycosides, beta lactam, quinolones, co- trimoxazole group and azithromycin [20]. The resistance of *V. cholera* to trimethoprim-sulfamethoxazole and erythromycin were 86% and 50% respectively, and this finding is similar to that recorded by Keramat *et al* [21] where the resistance rates were 98% and 62% for trimethoprim- sulfamethoxazole and erythromycin respectively. *V. cholera* was also found to be susceptible to cephalexine, nalidixic acid, gentamycin and fluoroquinolones [2] and this agrees with our results. Several resistance mechanisms such as plasmid encoded resistance, mutation in the quinolones resistance determine regions, intergrons and efflux pumps may be responsible for this resistance [11].

Antibacterial activity of crude methanol extract of *Q. infectoria* against isolates showed that no effect against all bacteria under study at concentration of 0.625 mg/ml and less of then. But more active against *E. coli* polyvalent I and IV result in inhibition zones 30mm of each of them and *E. coli II, III, Sh. desenterae* and *V. cholera* 15mm, *S. arizona* and *S. typhi* 10mm each of them in concentration 20.0mg/ml.

Antibacterial activity of methanol extract of *Q. infectoria* against *E. coli* I, II, III, IV, *Sh. desenterae*, *S. arizonae*, *S. typhi* and *V. cholera* revealed that the diameters of the inhibition zones were 21, 12, 12, 25, 14, 6, 7 and 13 mm respectively at a concentration of 10.0mg/ml.

The sizes of the inhibition zones of methanol extract of *Q. infectoria* against *E. coli* I, II, III, IV and *Sh. desenterae*, *S. arizonae*, *S. typhi* and *V. cholera* were 15, 1, 1, 25, 11, 5, 5, and 10

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mm respectively 5 mg/ml. There were no effects of this extract against *S. arizonae* and *S. typhi* at 2.5 mg/ml, there only one effect on *E. coli IV* (10 mm) at a concentration of 1.25 mg/ml. Our results are in agreement with Leela and Satirapipathkul [22] who found that *Q. infectoria* have antimicrobial activity against Gram-positive and Gram-negative bacteria. The antimicrobial effect of aqueous, methanol and ethanol extracts of *Q. infectoria* on the isolated bacterial species has been studied previously and found that the minimum inhibitory concentration (MIC) of ethanol extract on *E. coli* was 6.25 mg/ml [23] another study by Shariatifar *et al.* [24] showed that extracts from *Q. infectoria* showed good antimicrobial activity against food borne pathogens and the authors concluded that the extracts can be used in food preservation systems to inhibit the growth of these bacteria and improve food quality and safety. The MIC of the methanol extracts of *Q. infectoria* against different bacteria was ranging between 2.5 mg/ml and 20.0 mg / ml and the MBC was 1.25 mg/ml - 20.0 mg/ ml. *Q. infectoria* antibacterial property against common pathogens such as *Enterococcus faecalis*, *Streptococcus pyogenes*, and *Bacillus cereus* has been studied previously and the results showed that the MIC values against each bacteria species were ranging from 0.16 to 0.63 mg/ml and the methanol extract showed higher MBC value (0.31 mg/mL) [25].

Conclusions

The isolated bacteria showed high resistance to most widely used antibiotics and the methanol extract from *Q. infectoria* showed high potential as antibacterial agent.

Author contribution

Dr. Zirk Faki Ahmed Abdul Rahman, Dr. Beyman Akram Hama Said and conducted the study, collected the data and performed the diagnostic of bacteria and drafting of the article. Dr. Rana Mujahid Abdullah Al-Shwaikh contributed in the designing, organization and finalization of the protocol.

Conflict of interest

The authors declare no conflict of interest concerning this work.

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