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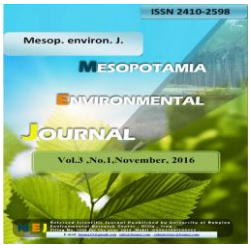

The antimicrobial activity of Al-Ankabut's home (Spider's web) extract

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Abstract

This study is the first in Iraq, in which using Al-Ankabut's home extract as antibacterial biomaterial. Different solvents were used to extract Al-Ankabut's home (distilled water, ethanol and acetone). These extracts were examined to determination of antibacterial activity against pathogen bacteria. The results of the present study shows the acetone extract has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 12 mm for *Enterobacter cloacae*, 8 mm for *Escherichia coli* and *Pseudomonas aeruginosa*, 10 mm for *Klebsiella pneumonia* and *Proteus mirabilis*, 14 mm for *Bacillus subtilis*, 16 mm for *Staphylococcus aureus*, and 12 mm for *Streptococcus spp.* The antimicrobial activity of distil water extract and ethanol extract of Al-Ankabut's home has no effect against tested isolates. The antimicrobial activity of distil water extract and ethanol extract of Al-Ankabut's home has no effect against tested isolates. The results of the effect of different concentration of acetone extract of the Ankabut's home against Gram negative and Gram positive bacteria shows the best concentration was 40 mg/ml, the inhibition zone was 12 mm for all Gram negative isolates. The inhibition zone was 14 mm for *Enterobacter cloaca*e, 12 mm for *Bacillus subtilis* and *Streptococcus spp.*, 17mm for *Staphylococcus aureus*. Whereas the 10 mg/ml concentration gives the lowest effect, the inhibition zone was 8 mm for *Klebsiella pneumonia*, *Enterobacter cloaca*e, *Bacillus subtilis* and *Streptococcus spp.* and 10 mm for *Staphylococcus aureus*. No effect against other Gram negative isolates. The study revealed that the acetone Al-Ankabut's home extract could be as a therapeutic agent for human microbial infections.

Keywords: Al-Ankabut's home extract, Antibacterial activity, Multi-drug resistance bacteria

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Introduction

Spider silk is a remarkable material in nature; showing levels of strength, torsionality, flexibility, lightness that are unmatched by man-made materials [1]. Another notable feature of spider silk is its longevity. Spider webs often remain in the environment long after the spider has expired. The longevity of spider silk indicates that the material has some properties of resisting decomposing by microorganisms. Studies by Vollrath *et al* have investigated the compounds present in spider silk and have found that spider silk contains molecules that are known to have antimicrobial properties [2]. Microbes are an incredibly diverse group of organisms but unfortunately some species have extremely detrimental effects on human health, society and economics. Many known antimicrobial compounds that exist have been very effective in combating and curing diseases from all over the world but currently untreatable diseases and the growing problem of resistant strains of microorganisms have made it more important than ever that cheap, reliable, antimicrobial compounds are discovered. Many of the currently used antimicrobial compounds have their origin in nature but research has focused on plant and fungi compounds while research on potentially antimicrobial animal compounds has been neglected. Because of spider silk is known not to provoke an immune response in humans [3], it is an excellent candidate for research on potentially very useful antimicrobials. Spiders use silk for a variety of different purposes such as web spinning, cocoon construction and as substrates upon which to deposit sperm. Silks vary in their mechanical properties such as in the degree of extensibility and maximum strength [4, 5]. The range of physical properties observed likely reflects differences amongst silk types in the relative proportions and combinations of particular amino acids such as alanine, glycine and proline [6]. Spider silks can be grouped into five basic categories: dragline (also known as major ampullate), capture spiral, tubiliform, aciniform and minor-ampullate silks, each of which is used for a different purpose. There are records indicating that spider silk has historically been used by humans for a variety of purposes. Peoples of the Carpathian Mountains are reported as using sections of the tubular shaped webs of Atypus spiders as topical bandages to heal wounds. This was believed to be beneficial due to the antiseptic properties of the spider silk [7]. Few studies, however, appear to have investigated this property in detail.

During the Hijrah, Migration of the Prophet Muhammed (sws) from Makkah to Madina in the year 622 A.D. One of the best trackers of the Quraish, a man named Abu Karz, traced the footprints of Muhammed, the Prophet of Islam (sws) to the cave of Thaur. However, when some men came near the mouth of the cave, they saw that its entrance was blocked by a spider's web and some wild pigeons had laid eggs in a nest at the entrance. The men knew that the spider and pigeons would not have made their homes there if there had been anyone in the cave. Also, if the web had been there from before, it would have been damaged if someone had entered the cave. They therefore returned without looking inside. By this miracle Allah (swt) protected His beloved messenger. This story described in The Holy Quran, Surah Al- Tawbah, Verse: 40 ((In the name of Allah, most gracious, most merciful, If you help him not, then *know that* Allah helped him *even* when the disbelievers drove him forth while he was one of the two when they were both in the cave, when he said to his companion, 'Grieve not, for Allah is with us.' Then Allah sent down His peace on him, and strengthened him with hosts which you did not see, and humbled the word of those who disbelieved, and it is the word of Allah alone which is supreme. And Allah is Mighty, Wise)) [8, 9].

Cobweb is also mentioned in the Holy Quran, Surah Al-Ankabut, Verse 41, which states ((In the name of Allah, most gracious, most merciful, The example of those who take allies other than Allah is like that of the spider who takes a home. And indeed, the weakest of homes is the home of the spider, if they only knew)) [10].

Therefore, In Iraq the spider its so-called Ankabut of the Prophet and the spider web called Al-Ankabut's home savior of the Messenger of Allah figure 1.

This study is the first in Iraq, in which they investigate Al-Ankabut's home (Spider's web) extract as antimicrobial biomaterial against multidrug resistance bacteria.

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Figure1. The entrance of the cave of Thaur was blocked by Al-Ankabut's home and some wild pigeons had laid eggs in a nest at the entrance.

Materials and methods

2.1. Collection of pathogens

We are collected multiple antibiotic-resistant isolates, which included *Enterobacter cloacae*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Streptococcus sp*., *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* used for the antimicrobial activity from microbiology diagnosis laboratory, Al-Numan hospital.

2.2. Al-Ankabut's home

Al-Ankabut's home used in this study was obtained from a residential building using a stick to collect the webs; silk was tested from one species of spiders, *Tegenaria Domestica*, figure 2. The webs were taken to the laboratory and washed thoroughly using distilled water to remove dust and other extraneous materials. The washed web was allowed to air-dry at room temperature (30 ± 2 ºC) and kept in air-tight container until further use.

2.3. Preparation of Al-Ankabut's home extract

The modified procedure was used [11]. One gram of Al-Ankabut's home was weighed and hydrolyzed with 10 ml of different solvents separately to extract sample (distilled water, ethanol and acetone) at 30ºC for one week. The hydrolyzed cobweb was centrifuged at 4000 rpm for 30 min. The supernatant obtained designated as Al-Ankabut's home extract. In the next step for ensure that is not contamination in the solution and generate errors in test, solution were passed through from filters with pores size 0.4 micrometer. The extract was stored in refrigerator in order to be used for further experiments figure 3.

2.4. Determination of antimicrobial activity

The antibacterial activity of Al-Ankabut's home extract was tested by standard agar well diffusion method [12]. Wells were made using sterile cork-borer under aseptic conditions. The inocula were prepared by diluting the overnight cultures with 0.9 % sodium chloride to 0.5 McFarland standards and were swabbed onto the plate. Al-Ankabut's home extracts were loaded on marked wells with the help of micropipette under aseptic conditions and incubated at 37 ºC for 24 h. The zone of inhibition was measured and expressed in millimeters.

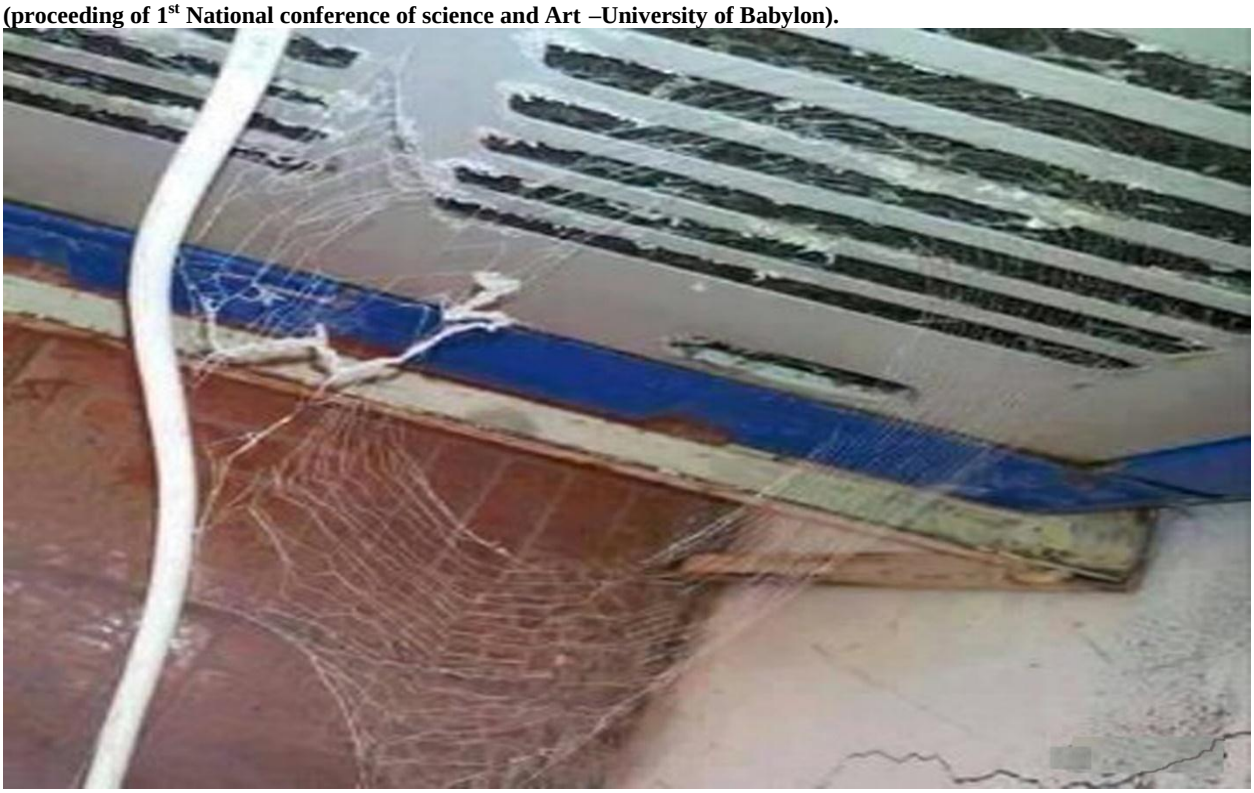

Figure 2. Shows Al-Ankabut's home on Air cooler.

Figure 3. Preparation of Al-Ankabut's home extract.

Results

The results show the acetone extract has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 12 mm for *Enterobacter cloacae*, 8 mm for *Escherichia coli* and *Pseudomonas aeruginosa*, 10 mm for *Klebsiella pneumonia* and *Proteus mirabilis.* 14 mm for *Bacillus subtilis*, 16 mm for *Staphylococcus aureus*, and 12 mm for *Streptococcus spp*. The antimicrobial activity of distil water extract and ethanol extract of Al-Ankabut's home has no effect against tested isolates table 1 and figures (4-6).

Table 1. The inhibitory activity of the Ankabut's home extracts against the tested bacteria as demonstrated by diameters of the inhibition zone (mm)*.

Isolated bacteria	Zone of Inhibition		
	(D.W) Al-Ankabut's home extract	(EtOH) Ankabut's home extract	(Ace.) Ankabut's home extract
Enterobacter cloacae	0	0	12
Escherichia coli	0	0	8
Klebsiella pneumonia	0	0	10
Proteus mirabilis	0	0	10
Pseudomonas aeruginosa	0	0	8
Bacillus subtilis	0	0	14
Staphylococcus aureus	0	0	16
Streptococcus spp.	0	0	12

* Zone of inhibition, including the diameter of the cup plate method (6.0 mm) .The recorded value is mean value of 3 replicates. D.W= distil water, EtOH= ethanol, Ace. = acetone.

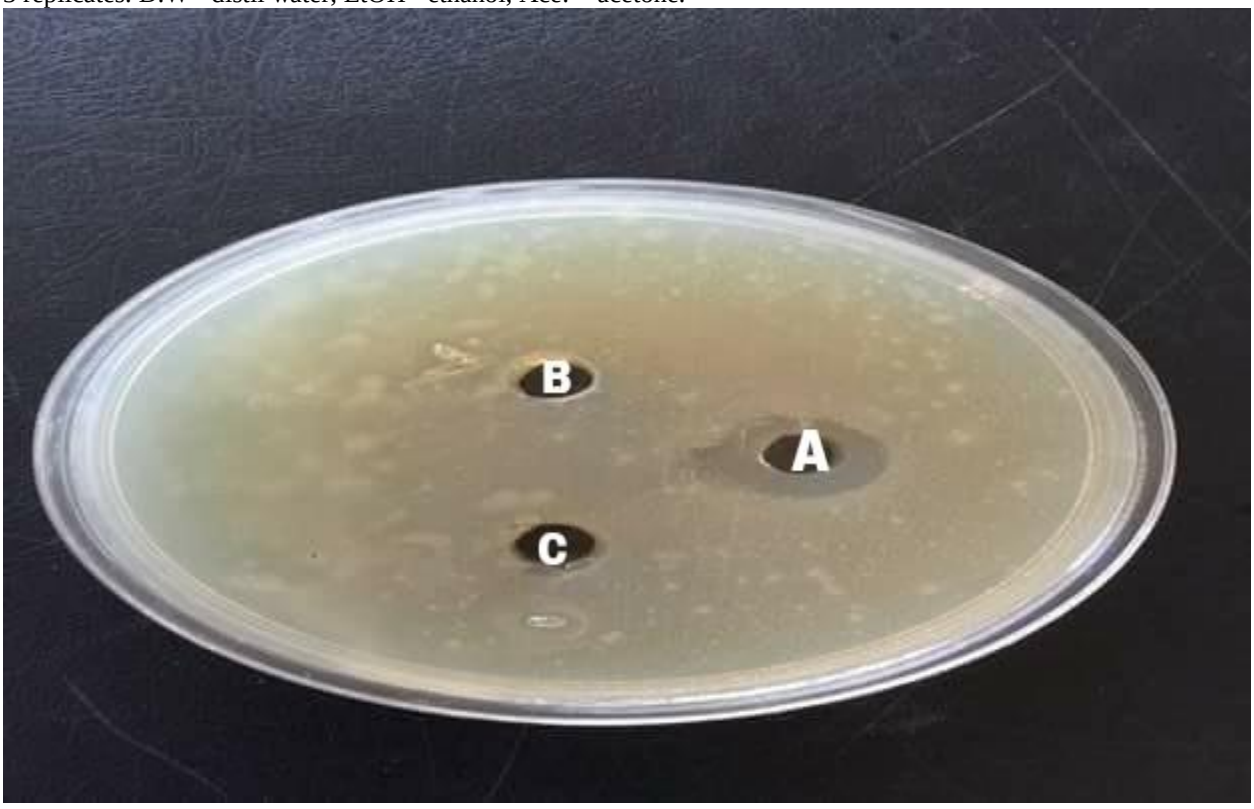

Figure 4. The antibacterial effects of the Ankabut's home extracts, acetone extract (A), ethanol extract (B) and distilled water extract (C), using the test bacterium *Proteus mirabilis*.

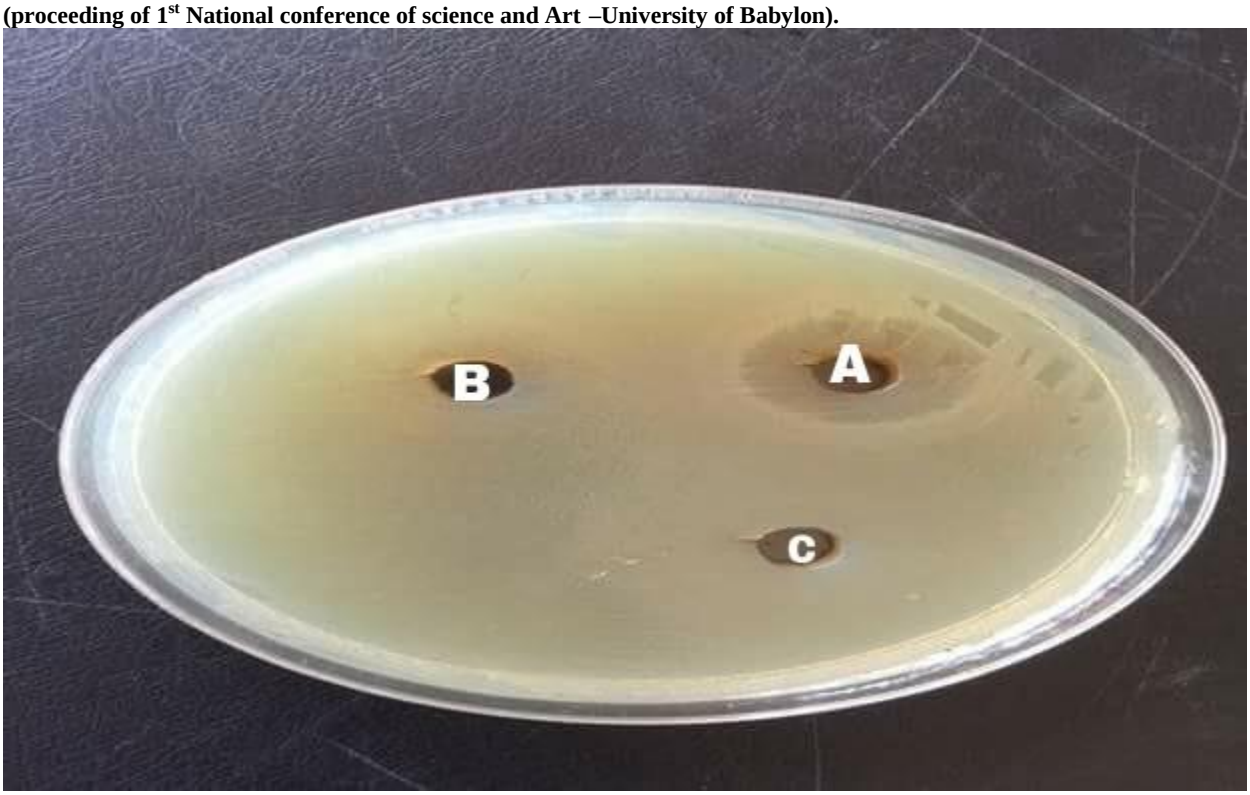

Figure 5. The antibacterial effects of the Ankabut's home extracts, acetone extract (A), ethanol extract (B) and distilled water extract (C), using the test bacterium *Staphylococcus aureus*.

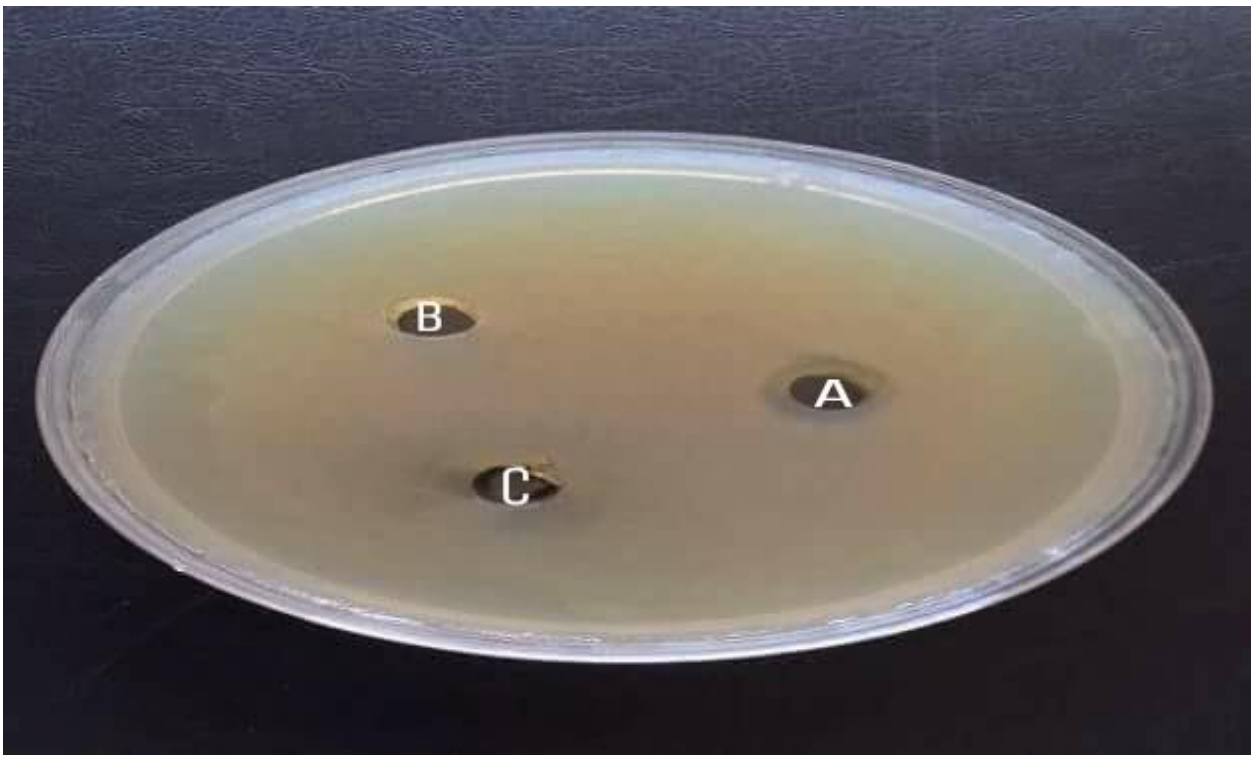

Figure 6. The antibacterial effects of the Ankabut's home extracts, acetone extract (A), ethanol extract (B) and distilled water extract (C), using the test bacterium *Escherichia coli*.

The results of the effect of different concentration of acetone extract of the Ankabut's home against Gram negative bacteria shows the best concentration was 40 mg/ml, the inhibition zone was 12 mm for all test isolates. Whereas the 10 mg/ml concentration gives the lowest effect, the inhibition zone was 8 mm for *Klebsiella pneumonia* and no effect against other isolates table 2 and figures 7.

Table 2. The Effect of different concentration of acetone extract of the Ankabut's home against Gram negative bacteria as demonstrated by diameters of the inhibition zone (mm)*

Concentration mg/ml	*Escherichia coli*	*Klebsiella pneumonia*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
10	0	8	0	0
20	8	10	8	8
30	10	10	10	8
40	12	12	12	12
50	10	10	8	10

* Zone of inhibition, including the diameter of the cup plate method (6.0 mm) .The recorded value is mean value of 3 replicates.

The results of the effect of different concentration of acetone extract of the Ankabut's home against Gram positive bacteria shows the best concentration was 40 mg/ml, the inhibition zone was 14 mm for *Enterobacter cloaca*e, 12 mm for *Bacillus subtilis* and *Streptococcus spp.*, 17mm for *Staphylococcus aureus*. Whereas the 10 mg/ml concentration gives the lowest effect, the inhibition zone was 8 mm for *Enterobacter cloaca*e, *Bacillus subtilis* and *Streptococcus spp.* and 10 mm for *Staphylococcus aureus* table 3 and figures 8.

Table 3. The Effect of different concentration of acetone extract of the Ankabut's home against Gram positive bacteria as demonstrated by diameters of the inhibition zone (mm)*

Concentration mg/ml	*Enterobacter cloaca*e	*Bacillus subtilis*	*Staphylococcus aureus*	*Streptococcus spp.*
10	8	8	10	8
20	11	10	12	8
30	12	10	15	10
40	14	12	17	12
50	11	10	15	10

* Zone of inhibition, including the diameter of the cup plate method (6.0 mm) .The recorded value is mean value of 3 replicates.

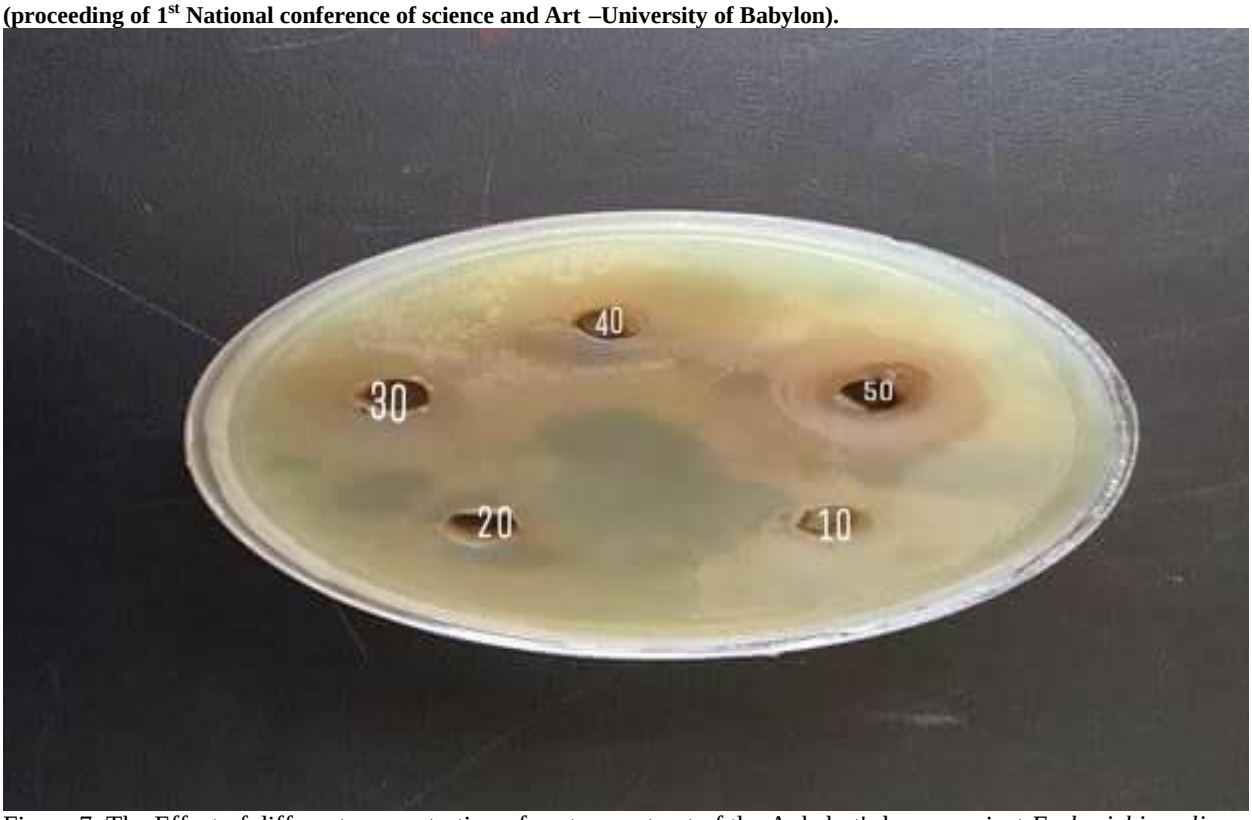

Figure 7. The Effect of different concentration of acetone extract of the Ankabut's home against *Escherichia coli*.

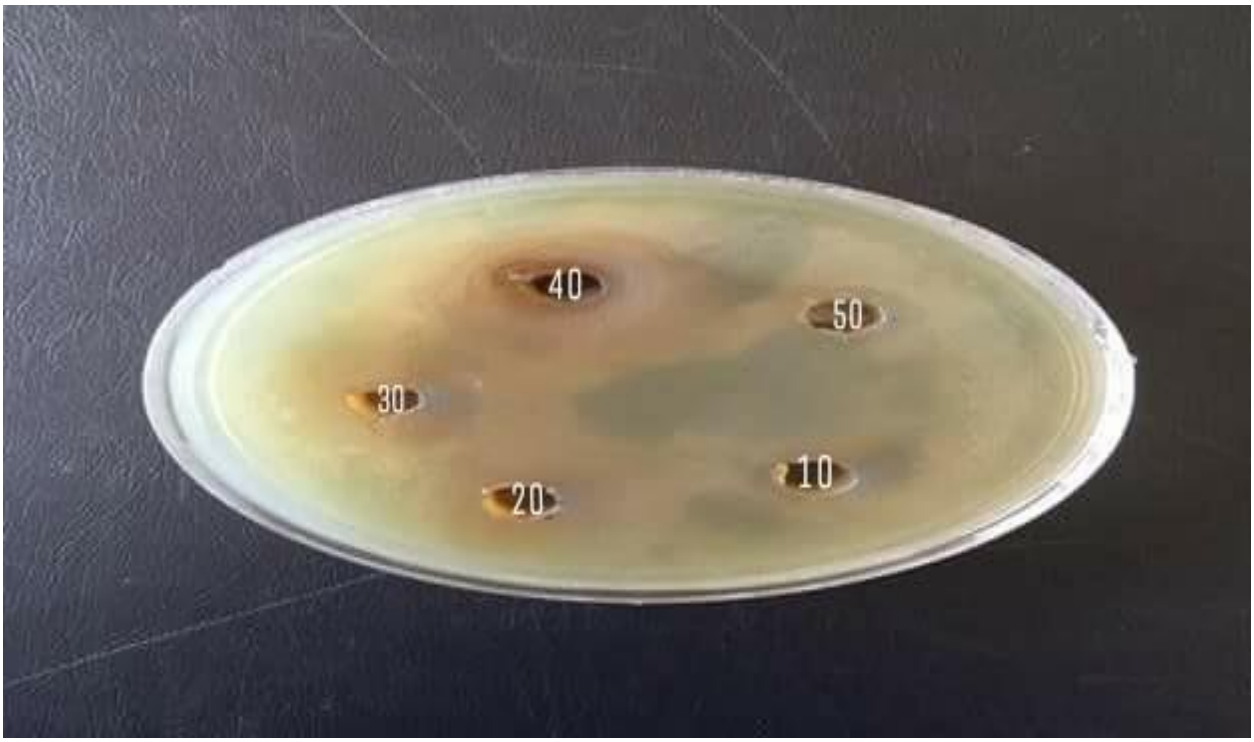

Figure 8. The Effect of different concentration of acetone extract of the Ankabut's home against *Enterobacter cloacae*.

Discussion

Al-Ankabut builds their home with a material called silk or web. Al-Ankabut's silk or Al-Ankabut's web contain protein fiber that have many advantages and functions. One of them is to capture their prey such as flies, insects, and others. The needs on the research of antibacterial activity are important for human health because of importance of finding a new cure for some diseases that occur because of microorganisms. Some of the microorganisms, especially bacteria are becoming resistant to many antibacterial agents. Therefor the need it's become an urgent to find therapeutic alternative substances to antibiotics. In this study we investigated the possibility of Al-Ankabut's home to contain antibacterial substances. Silk was tested from one species of spiders, *Tegenaria Domestica,* because previous studies refer to the silk of this type have antimicrobial activity against bacteria and fungi [13].

The results show the acetone extract has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 12 mm for *Enterobacter cloacae*, 8 mm for *Escherichia coli* and *Pseudomonas aeruginosa*, 10 mm for *Klebsiella pneumonia* and *Proteus mirabilis.* 14 mm for *Bacillus subtilis*, 16 mm for *Staphylococcus aureus*, and 12 mm for *Streptococcus spp*. The antimicrobial activity of distil water extract and ethanol extract of Al-Ankabut's home has no effect against tested isolates table 1 and figures (4-6). The antimicrobial activity of distil water extract and ethanol extract of Al-Ankabut's home has no effect against tested isolates. The results of the effect of different concentration of acetone extract of the Ankabut's home against Gram negative and Gram positive bacteria shows the best concentration was 40 mg/ml, the inhibition zone was 12 mm for all Gram negative isolates. The inhibition zone was 14 mm for *Enterobacter cloaca*e, 12 mm for *Bacillus subtilis* and *Streptococcus spp.*, 17mm for *Staphylococcus aureus*. Whereas the 10 mg/ml concentration gives the lowest effect, the inhibition zone was 8 mm for *Klebsiella pneumonia*, *Enterobacter cloaca*e, *Bacillus subtilis* and *Streptococcus spp.* and 10 mm for *Staphylococcus aureus*. No effect against other Gram negative isolates table 2and 3, figures (7-8).

Al-Ankabut's home had significant potential as an antibacterial compound. Acetone which is the less polarity shows the best antibacterial activity. Gram positive bacteria are higher inhibition zone compared to gram negative bacteria. Our interpretation of these results, the Al-Ankabut's home contained substance such have antimicrobial activity, these finding are in agreement with previous study in which, fond the spiders web have several peptides and proteins known with antimicrobial activity, most with broad activity against different microorganisms, including Gram positive and Gram negative bacteria, virus, protozoa and fungi [14]. The bactericidal activity of these molecules starts with direct binding to the lipid bilayer forming the bacteria membrane. After this interaction with the membrane the bactericidal peptides acquire an amphiphilic three-dimensional conformation where the positive side of the antimicrobial molecules interacts directly with the negatively charged lipid head-groups. This interaction results in the formation of pores through the bacterial membrane [15, 16, 17].

Conclusions

This research indicates that Al-Ankabut's home acetone extract is effective against both gram-positive and gram negative bacteria. Further research is currently underway to try and identify the substance in the Al-Ankabut's home extract that is inhibiting the growth of the microbes.

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