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Study of Antibacterial Activity from Alcoholic Extracts of *Torilis arvensis* (Huds.) Link on Biofilm formation of *Acinetobacter baumannii* in Diyala Province - Iraq

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Abstract

This study was conducted in Baqubah Education Hospital in Diyala/Iraq from the period 1st January to 2nd of March 2022. One hundred twenty clinical specimens from wounds and burns swab of different ages and gender and were obtained and cultured on suitable media (Blood agar and MacConkey agar) for isolation and identification of *Acinetobacter baumannii* according to standard bacteriological techniques incubated under suitable conditions. The current study was investigated for antibiotic susceptibility test, Minimum, Sub Inhibitory concentration (MIC and SUB-MIC) of antibiotics, biofilms formation, antibacterial effect of Methanol and ethanol extracts of *Torilis arvensis* (Huds.) link and active compounds of extract. Under current study, twenty isolates of *A.baumannii* were identified and which were distributed as (65 %) from wounds infections and 35% from burns infections. The Minimum Inhibitory Concentration of Meropenem and Levofloxacin ranged from 8-512 $ug.m^{-l}$. Antibiotics sensitivity test of bacteria revealed highly resistance for all antibiotics which were used in current study where the percentage value range from 46% for Tobramycin to 90% for

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Ampicillin-sulbactam and Ceftazidime, Isolates of *A.baumannii* showed high ability for biofilm formation with a ratio about 85 %. And 20 substances that were present in plant extract were detected by Gas Chromatography-mass spectrometry and recorded as active compounds. Ethanol and methanol extracts of *Torilis arvensis* (Huds.) Link demonstrated high antibacterial activity against *A. baumannii* isolates as concentration about 200 $mg.ml^{-1}$. The results of the current study showed that the stimulation of *A.baumannii* isolates for biofilm formation may be affected by the both SUB-MIC of antibiotics and Torilis arvensis (Huds.) Link.

Keywords: *Acinetobacter baumannii*; Biofilm formation; *Torilis arvensis*; Antibiotics

دراسة الفعالية المضادة للبكتيريا للمستخلصات الكحولية من نبات القميلة الحقلية *Torilis arvensis* (Huds.) Link على الغشاء الحيوي للبكتيريا البومانية في محافظة ديالى - العراق

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الخلاصة

اجريت هذه الدراسة في مستشفى بعقوبة التعليمي في ديالى / العراق للمدة من 1 كانون الثاني إلى 2 أذار 2022. تم جمع مائة وعشرون عينة سريرية من الجروح والحروق من مختلف الأعمار والجنس زرعت على الأوساط الغذائية الملائمة كما تم حضنها في ظل ظروف مناسبة لغرض عزل البكتريا الراكدة البومانية *Acinetobacter baumannii*، تم في هذه الدراسة اختبار الحساسية للمضادات الحيوية، التركيز المثبط الأدنى للمضادات الحيوية، تكوين الأغشية الحيوية، والتأثير المضاد للبكتيريا بوساطة الميثانول والايثانول المستخلصة من نبات القميلة الحقلية (*Torilis arvensis* (Huds وتحديد المركبات الفعالة في المستخلص. تم من خلال الدراسة الحالية عزل وتشخيص 20 عزلة من البكتريا الراكدة البومانية والتي توزعت على نحو 65% من اصابات الجروح و35% من اصابات الحروق، بينما تراوح الحد الأدنى للتركيز المثبط لمضادي ميروبنيم و ليفوفلوكساسين من 8-512 ميكروغرام. مل$^{-1}$. أظهر اختبار حساسية المضادات الحيوية وجود مقاومة

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عالية لجميعها اذ تراوحت القيمة المئوية 46% بالنسبة لمضاد توبراميسين إلى 90% لكل من الأسيسيلين - سولباكتام وسيفتازيديم. اظهرت العزلات قدرة عالية على تكوين الغشاء الحيوي بنسبة 85%، كما تم الكشف عن 20 مادة كانت موجودة في المستخلصات النباتية بواسطة مطياف الكتلة اللوني للغاز وتم تسجيلها كمركبات نشطة. كما أظهرت مستخلصات الإيثانول و الميثانول من *Torilis arvensis* (Huds.) Link تاثير تثبيطيا ضد عزلات الراكدة البومانية بتركيز 200 ملغم. مل$^{-1}$. واخيرا بينت نتائج الدراسة الحالية، ان درجة تحفيز عزلات البكتريا البومانية على تكوين الغشاء الحيوي قد يتأثر الى حد ما بمقدار الحد الادنى للتركيز المثبط للمضادات الحيوية من جهة وكذلك تركيز المستخلص الكحولي لنبات القميلة الحقلية *T. arvensis* من جهة اخرى

الكلمات المفتاحية: الراكدة البومائية، تكوين الغشاء الحيوي، مستخلصات *Torilis arvensis*، المضادات الحيوية

Introduction

One of the most common adverse events in healthcare is nosocomial infection/hospital-acquired illness [1]. Every year, about 5 million people in Europe are thought to be infected by hospital acquired infections, with a prevalence of 7.2 percent [2]. Hospital- acquired illnesses are caused by a variety of pathogens, which change depending on the country, patient demographic, and healthcare facility [3]. Bacteria, viruses, and fungi are all capable of causing HAIs, however, bacteria are the most prevalent causal organism [4].

Acinetobacter baumannii is one of the most dangerous drug-resistant bacteria on the planet [2]. The World Health Organization recently declared drug-resistant A. baumannii to be the priority pathogen, requiring urgent research and development of novel antibiotics [5]. Many dangerous diseases affect infected patients with resistant A. baumannii , including pneumonia septicemia, and urinary tract infections [6]. One of the difficult paths in the development of multidrug-resistant A. baumannii is biofilm production by bacterial cells which could explain antimicrobial resistance and abiotic surface survival in the presence of disinfectants and/or desiccants [7]. The global health community is concerned about the establishment and spread

of antibiotic resistance, as well as the evolution of new strains of disease-causing organisms [8]. The creation of new medicines or some prospective source of innovative therapeutics is required for effective illness therapy, Our community's commonly utilized medicinal herbs could be a good source of medications to combat this problem [9]. Bioactive compounds, notable polyphenols, are abundant in plant extracts, polyphenols suppress microorganisms, notably bacteria and they can change the shape of microorganisms, damage bacterial cell walls, and influence biofilm development [10]. The Apiaceae (Umbelliferae) family, also known as the carrot or parsley family, is one of the world's largest angiosperm plant groups and There are 300–462 genera and 2,500–3,750 species in the family [11]. For hundreds of years, Torilis arvensis (Huds.)A link has been used as a natural remedy to battle several ailments, including bacteria, fungi, and viruses, as part of the Apiaceae family [12,13]. The current study aimed to determine the effect of SUB-MIC of alcoholic extracts of T. arvensis and antibiotics against strong biofilms formation by A. baumannii isolates.

Materials and Methods

Samples Collection

One hundred and twenty specimens were collected from different clinical sources (wounds and burns) swab from patients of diverse gender and age at Baqubah Education Hospital in Diyala/Iraq from period 1st of January to 2nd of March 2022. After collecting, all the specimens were cultured on Blood agar and MacConkey agar purchased from mast group . All specimens were diagnosed by using biochemical tests (catalase and oxidase tests) and Vitek 2 system (BioMerieux) was done in educational laboratories at Baqubah Education Hospital [14].

Antibiotics Susceptibility Test

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The sensitivity test technique was done according to the Clinical and Laboratory Standards Institute [15]. Mueller-Hinton agar plates were used for the use of quickly growing species in the Kirby- Bauer method [16]. The antibiotics were used in this study are Piperacillin (PI), Ampicillin-sulbactam (AMS), Ceftazidime (CAZ), Cefotaxime (CTX), Tetracycline (TE), Levofloxacin (LEV), Gentamicin (GM), Amikacin (AK), Meropenem (MEM), Imipenem (IPM), Tobramycin (TOB) and Trimethoprim- sulfamethoxazole (STX). Meropenem 10 $ug.m^{-1}$ and Levofloxacin 5 $ug.m^{-1}$ were selected as antibiotics for the MIC measurement in all isolates of *A. baumannii* by using 96-well microplates. The reading of outcomes by ELISA (Kevin) reader on 630 nm wavelength [16] and this test was done in educational laboratories at Baqubah Education Hospital.

Detection of biofilm formation

The microtiter plate (also called 96-well plate) assay was used for studying biofilm formation, a method that allows for the observation of bacterial adherence to an abiotic surface [17]. The results ELISA reader was used to measure the Optical Density OD by wave length 630 nm. Biofilm production was classified as negative, weak, moderate, and strong based on the cutoff value, calculated according to the following formula, using the optical density (OD) values [17]:

OD cutoff = ODavg of negative control + (3 × standard deviation of ODs of negative control).

The used criteria were as follows:

i. $OD \leq ODcutoff$ = Non-biofilm former
ii. $ODcutoff < OD \leq 2 \times ODcutoff$ = Weak biofilm former
iii. $2 \times ODcutoff < OD \leq 4 \times ODcutoff$ = Moderate biofilm former
iv. $OD > 4 \times ODcutoff$ = Strong biofilm former

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Preparation of alcoholic extracts of Torilis arvensis

Ten grams of Torilis arvensis (Huds.) Link leaves were dissolved in 100 mL absolute methanol and 70% ethanol in a vibrating incubator for 24 hours at 34 °C and then the liquid was centrifuged for 15 minutes at 3000 RPM [18]. The extract was dried in an oven at 30-40 degrees Celsius and then placed in sterilized glass bottles and stored in the refrigerator until needed.

Detection of active compounds in plant extract

Active substances and compounds have been detected in the plant extracts of T. arvensis (Huds.)Link with a Gas chromatography-mass spectrometry (GC-MS mass spectrometry) at the Department of Environment and Water / Environmental Research Center at the Ministry of Science and Technology and Ibn Al-Bitar Center of the Ministry of Industry and Minerals. It is noteworthy to say that this is an effective method for the Separation and detection of volatile organic compounds and gaseous mixtures of various inorganic compounds [19].

Determination of the antibacterial activity of alcoholic extracts of Torilis arvensis plant

Agar well diffusion method was used to determine the effect of alcoholic extracts of T. arvensis (Huds.)Link against clinical bacterial isolates according to [20] with some modifications. Three concentrations were used in this method as follows (50, 100, and 200) mg.ml -l. The positive control was represented by adding methanol only, three replicates worked for each dish, and finally, the plates incubate at 37 °C for 24 h.

Effects of alcoholic extracts of Torilis arvensis on the strong biofilm formation

The microtiter plate's method was used to study the effect of alcoholic extracts on bacterial isolates that only produced strong biofilm formation according to [21]. The clinical isolates were cultured on brain heart infusion broth and incubated at 37°C for 24 hours. After

incubation, the broth cultures were compared with a MacFarland standard No. 0.5. Different concentrations of methanol and ethanol extracts were used in this method (50, 100, 200) mg.ml -l, and then 200 µl of concentrations were placed in each hole in the microtiter plate while the only methanol and ethanol were used as control.

Results and Discussion

Isolation and Diagnosis of Acinetobacter baumannii

The number of isolates of A. baumannii were obtained from a total of 120 samples including20 bacterial isolates with a percentage of 29.1% were collected from different clinical sources as follows in figure 1: wounds 65% while burns 35%. Biochemical tests showed that A. baumannii were positive for the catalase test and negative for the oxidase test. VITEK 2 system was used to confirm that all clinical isolates belong to A. baumannii.

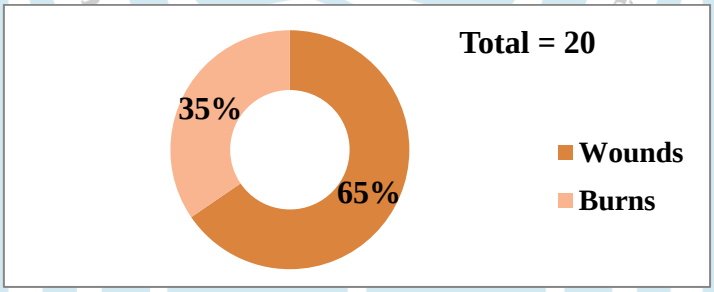

Figure 1: The percentage isolates of Acinetobacter baumannii according to sources

The clinical isolates of A. baumannii obtained from wounds and burns were 13/20 65% and 7/20 35% correspondingly, according to the results of figure 1. Our findings were compatible with studies that were done in Iraq [22, 23]. In a study carried out by [24] in which 26 isolates of A. baumannii were diagnosed, as 62 percent of wound isolates and 36 percent clinical isolates from burns these findings were similar to our results. The frequency of multidrug resistant

clinical isolates of A. baumannii which were obtained from burns and wounds has recently increased by 91%, the high incidence of A. baumannii isolation is connected to several causes, including A. baumannii acquisition [25].

Antibiotics susceptibility test (AST)

Antibiotics susceptibility test showed that most clinical isolates of Acinetobacter baumannii have high resistance to antibiotics. Figure 2 showed A. baumannii resistance to antibiotics as follows: Piperacillin 84%, Ampicillin-sulbactam 90%, Ceftazidime 90%, Cefotaxime 85%, Tetracycline 79%, Levofloxacin 75%, Gentamicin 82%, Amikacin 81%, Meropenem 85%, Imipenem 80%, Tobramycin 46% and Trimethoprim- sulfamethoxazole 65%. The MIC (Levofloxacin and Meropenem) of all isolates ranged from 8 to 512 table 1.

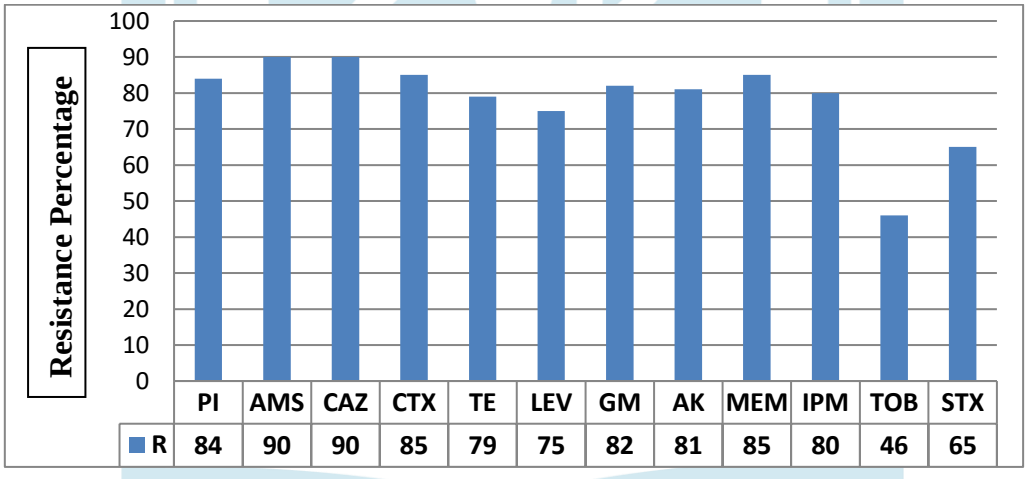

Figure 2: Antibiotics resistance of *A. baumannii* isolates

R- Resistance, Piperacillin (PI), Ampicillin-sulbactam (AMS), Ceftazidime (CAZ), Cefotaxime (CTX), Tetracycline (TE), Levofloxacin (LEV), Gentamicin (GM), Amikacin (AK), Meropenem (MEM), Imipenem (IPM), Tobramycin (TOB) and Trimethoprim- sulfamethoxazole (STX).

Table 1: The MIC values (µg/ml) of antibiotics against *A. baumannii*

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NO. OF THE ISOLATES	LEV MG.ML^{-1} MIC	MEM MG.ML^{-1} MIC	NO. OF THE ISOLATES	LEV MG.ML^{-1} MIC	MEM MG.ML^{-1} MIC
A1	64	512	A11	256	128
A2	8	16	A12	256	512
A3	256	32	A13	64	64
A4	128	256	A14	512	128
A5	512	128	A15	64	128
A6	256	64	A16	16	32
A7	128	256	A17	32	8
A8	128	32	A18	512	32
A9	16	8	A19	256	64
A10	512	512	A20	32	128

The Antibiotics Susceptibility test (AST) in Fig 2 showed *A. baumannii* has high resistance to antibiotics, the sub-mic of Levofloxacin showed high effect against the no. 2 isolate of *A. baumannii*, in contrast, the isolate of no.18 showed high resistance against the concentration of Levofloxacin was 512 µg.ml-1, biofilm formation one of the important reasons which give high resistance against antibiotics. When comparing the current results with other studies which were done in Iraq, the researchers [22, 23] founds the antibiotic resistance against Piperacillin, Ceftazidime, Cefotaxime, Meropenem, Imipenem, Amikacin and Levofloxacin were 95%, 90%, 90%, 85%,95%, 90%, 95% and 100%, 100%, 100%,90%,90%, 95%, 95% respectively, these results were close to present study. The clinical isolates in this investigation revealed substantial resistance to ampicillin-sulbactam and tetracycline 90 % and 70%, respectively.

These findings contradict a local study that found *A. baumannii* resistance to (Ampicillin-sulbactam) was 40% [22]. On the other hand, [26] revealed that *A. baumannii* isolates were resistant to Tetracycline in 78 percent and 65 percent of cases, respectively, which agrees with our findings but differs from [27] who identified a rate of Tetracycline resistance of 20%. Tetracyclines and Glycylcyclines block aminoacyl tRNA from attaching to the target ribosome,

therefore inhibiting protein synthesis [28]. The variation in resistance could be attributed to sampling sources, as well as environmental and test conditions.

The Minimum inhibitory concentration (MIC) of Meropenem and Levofloxacin of 20 isolates *A. baumannii* were shown in table 1, that ranged from 8 to 512. Carbapenem antibiotics such as meropenem belong to the ß-lactam family and remain active against most ß-lactamase-producing organisms, including those with extended spectrum ß-lactamase enzymes [29]. Quinolones are bactericidal with a broad spectrum that is characterized by a bicyclic core formation bearing resemblance to 4-quinolone. Quinolone antibiotics are mostly fluoroquinolones (Levofloxacin) displaying efficacy against both Gram-negative and Gram-positive pathogens [30].

Biofilm formation of *Acinetobacter baumannii*

All isolates of *A. baumannii* were evaluated based on their ability to biofilm formation using the microtiter plate method. The results of the current study presented in figure 3 showed that the majority of *A. baumannii* isolates which produced biofilm as Strongly, Moderately, Weak and non- biofilm producers were 55%, 25%, 5%, 15% respectively.

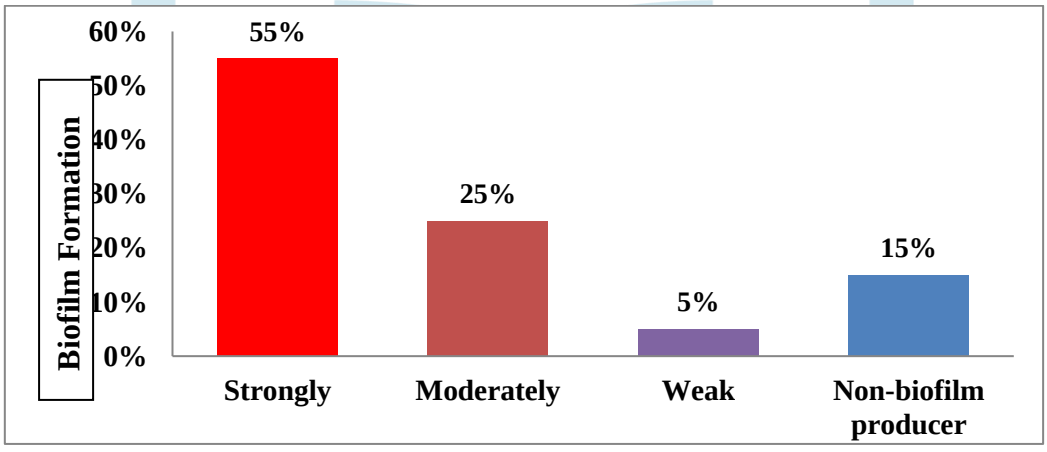

Figure 3: The percentage Biofilm formation of *A. baumannii* isolates

The results shown in Fig 3 were close to studies done in Iraq [22,23] which founds that most isolates of *A. baumannii* had an ability to strong biofilm formation. Many bacterial diseases, including *A. baumannii*, can produce biofilms, which are matrix contained communities. The ability of *A. baumannii* isolates to form biofilms may have played a key role in the survival and persistence of bacterial infection in the presence of environmental stress factors [31].

Gas chromatography-mass spectrometry Analysis

GC-MS mass spectrometry was used to determination of active compounds in plant extract of *T. arvensis*. Gas chromatography is a chemical analytical instrument for the separation and characterization of chemicals in the sample. The active compounds found in *T. arvensis* (methanol and ethanol) extracts were showed in table 2.

Table 2: showed the active compounds, peak and area of *Torilis arvensis*

NO.	ACTIVE COMPOUNDS	PEAK	AREA	MAX%	TOTAL
1	Propanoic acid, 3-hydroxy-, methyl ester	12639	171073	0.48%	0.307%
2	1,4-Butanediamine	16023	125467	0.35%	0.226%
3	5-Aminovaleric acid	13969	128558	0.36%	0.231%
4	Acetic acid, (1-methylethoxy)-, ethyl ester	12161	83184	0.23%	0.150%
5	2-Butyl(dimethyl)silyloxybutane	159649	3900783	10.98%	7.011%
6	2-Dimethyl(octyl)silyloxybutane	15272	91078	0.26%	0.164%
7	Silane butoxytrimethyl	203366	7339302	20.65%	13.192%
8	Glucuronamide	584635	35542216	10.00%	63.884%
9	O-glycosides	77506	832711	2.34%	1.497%
10	L-Alanine, 3(aminocarbonyl)amino	49942	869726	2.45%	1.563%

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11	Terpenoids	52724	443979	1.25%	0.798%
12	5-Cyano-3-ethoxycarbonyl-1,2,3,4-t etrahydro-4,6-dimethyl-2-oxopyridine	27643	240425	0.68%	0.432%
13	1-Diphenyl(tert-butyl) silyloxy-4-m ethylbenzene	133617	2054243	5.78%	3.692%
14	Butanoic acid, ethyl ester	16388	89762	0.25%	0.161%
15	N-Acetyl-D-glucosamine	30188	154826	0.44%	0.278%
16	8-Octadecynoic acid, methyl ester	114454	2884298	8.12%	5.184%
17	Apigenin	15977	107903	0.30%	0.194%
18	Oleic Acid	16027	122003	0.34%	0.219%
19	Hexacosanoic acid	12710	86649	0.24%	0.156%
20	1-(methylene-1-trimethylsilylcyclo propyl)-	34361	366943	1.03%	0.660%

The active compounds found in *T. arvensis* extracts was showed in the table 2. 2-Butyl(dimethyl)silyloxybutane and glucuronamide were the highest percentage compounds found in *T. arvensis* extracts with 7.011% and 63.884%, respectively, these compounds play important role in effect against biofilm formation of *A. baumannii* isolates through the effect on cell wall of bacteria. The majority of T.*arvensis* phytochemical research has concentrated on terpenoids [32], hence data on flavonoids is scarce. They looked at the flavonoids in the leaves of over 300 Apiaceae species and only identified luteolin-7-glucoside in a few Torilis taxa [33]. In *T. arvensis*, [34] discovered luteolin and apigenin glycosides (glucoside or glucuronide). For detecting flavonoids in plant extracts, mass spectrometry combined with liquid chromatography (LC/MS), tandem mass spectrometry (MS/MS), and gas chromatography-mass spectrometry (GC-MS) is useful instruments. They're typically combined with electrospray ionization (ESI) to determine flavonoid concentration in raw extracts [35].

Antibacterial activity of the methanol extract of Torilis arvensis against Acinetobacter baumannii

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The results of the current study shown in figure 4 and 5 showed that methanol and ethanol extracts of *T. arvensis* (Huds.)A link affects the isolates of multidrug resistance (MDR) of *A. baumannii* . The concentration at 200 mg.ml^{-1} of methanol and ethanol extracts showed a high ratio 49% and 40% against isolates of *A. baumannii* followed by a concentration at 100 mg.ml^{-1} of 35% and 30% while at 50 mg.ml^{-1} showed a lower effect by 15% and 11% respectively.

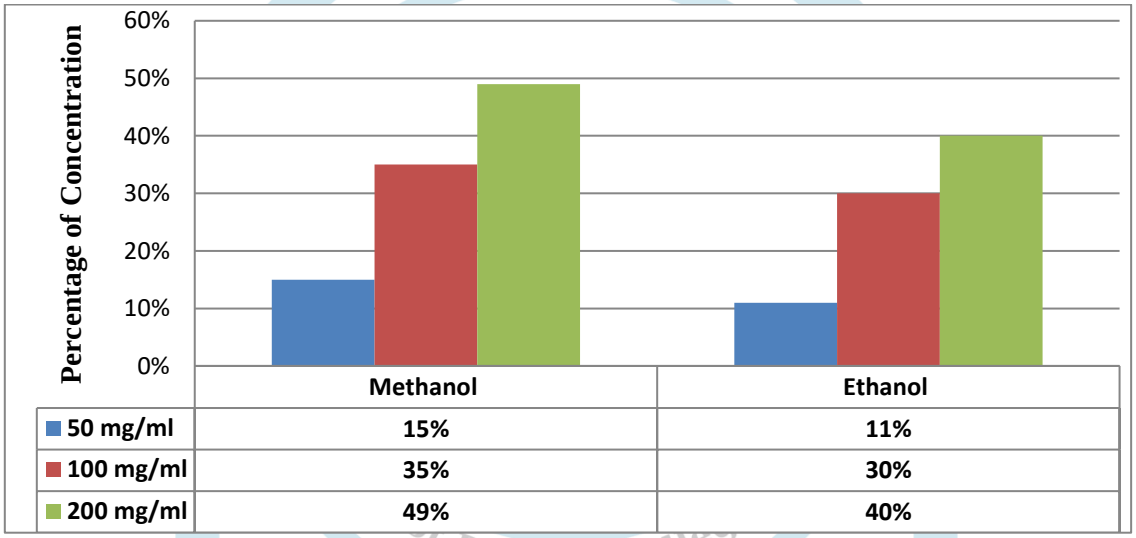

Figure 4: The Percentage of *Torilis arvensis* extracts on (15 isolates) of MDR *A. baumannii*

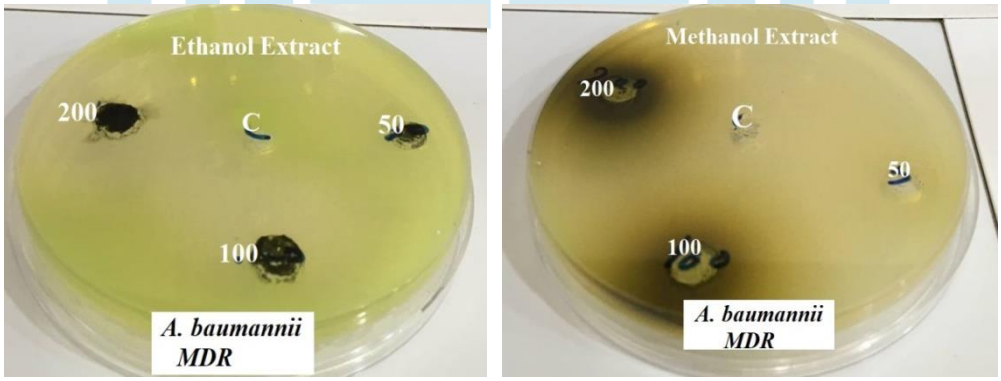

Figure 5: Antibacterial activity of the alcoholic extracts of Torilis arvensis against MDR of *A. baumannii*

The results in Figure 4 and 5 showed the methanol and ethanol extracts of *T. arvensis* have antibacterial activity against MDR isolates of *A. baumannii*, the concentration at 200 mg/ml of both extracts have a high ratio against isolates of *A. baumannii* than 100 $mg.ml^{-1}$ and 50 $mg.ml^{-1}$. Several studies agree with our results, the extract of Torilis spp. have antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans* [36]. Antimicrobial activity of Torilis sp. oil was tested against three standard bacteria strains (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and two standard fungus strains (*Candida albicans* and *Pichia guilliermondii*) The oil displayed moderate effectiveness against the infections tested in vitro, according to the findings [37].

Effects of alcoholic extracts of *Torilis arvensis* and antibiotics on the strong biofilm formation

Eleven isolates of *A. baumannii* was strong biofilm formation that was reduced after treatment with sub-antibiotics (Meropenem and Levofloxacin) and sub-alcoholic extracts (Tables 3 and 4). The biofilm of most *A. baumannii* isolates decreased from strongly to moderately and non-biofilm producer, the isolates that decreased in biofilm formation are A1, A3, A5, A6, A8, A10, A12, A14, A15, A18, A20.

Table 3: The effect of SUB-MIC antibiotics on Strong biofilms formation by *A. baumannii*

ISOLATES NO.	AFTER MEROPENEM TREATMENT			AFTER LEVOFLOXACIN TREATMENT		
	Sub MIC	Absorbency at 630 nm	Biofilms formation	Sub MIC	Absorbency at 630 nm	Biofilms formation
A1	256	0.042	Non-biofilm producer	32	0.076	Moderately
A3	16	0.115	Strongly	128	0.128	Strongly
A5	64	0.075	Moderately	256	0.081	Moderately

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A6	32	0.044	Non-biofilm producer	128	0.076	Moderately
A8	16	0.077	Moderately	64	0.047	Non-biofilm producer
A10	256	0.083	Moderately	256	0.070	Moderately
A12	256	0.123	Strongly	128	0.081	Moderately
A14	64	0.069	Moderately	256	0.114	Strongly
A15	64	0.073	Moderately	32	0.073	Moderately
A18	16	0.083	Moderately	256	0.133	Strongly
A20	64	0.085	Moderately	16	0.084	Moderately

Table 4: The effect of *Torilis arvensis* extracts (SUB-MIC) on Strong biofilms formation by *A. baumannii*

ISOLATES NO.	AFTER METHANOL TREATMENT (SUB-MIC 100)		AFTER ETHANOL TREATMENT (SUB-MIC 100)	
	Absorbency at 630 nm	Biofilms formation	Absorbency at 630 nm	Biofilms formation
A1	0.042	Non-biofilm producer	0.069	Moderately
A3	0.070	Moderately	0.044	Weak
A5	0.077	Moderately	0.085	Moderately
A6	0.038	Non-biofilm producer	0.050	Weak
A8	0.040	Non-biofilm producer	0.041	Non-biofilm producer
A10	0.043	Non-biofilm producer	0.047	Non-biofilm producer
A12	0.045	Non-biofilm producer	0.043	Non-biofilm producer
A14	0.072	Moderately	0.041	Non-biofilm producer
A15	0.069	Moderately	0.052	Weak
A18	0.042	Non-biofilm producer	0.051	Weak
A20	0.046	Non-biofilm producer	0.042	Non-biofilm producer

Tables 3 and 4 showed the sub-mic after treatment with meropenem, levofloxacin, methanol, and ethanol extracts of *Torilis arvensis* decreased strong biofilm formation. The extracts *Torilis arvensis* did not effect on the isolates of no. 3,12 and 18 because these isolates have high strong biofilm formation and also may have many virulence factors which making it XDR against antibiotics and extracts while the isolates no. 1,6 and 8 which became inability to form biofilm formation after treatment with *Torilis arvensis* extracts. *Torilis arvensis* extracts shown rich active compounds showed in table 2 especially -Butyl(dimethyl)silyloxybutane and

glucuronamide Which is attributed to the ability to affect the biofilm of *A. baumannii* in addition to other compounds.

A study was done by indicated the inhibitory activity of ethanolic and methanolic extracts of Apiaceae against *Staphylococcus aureus, Bacillus cereus, E. coli, Listeria monocytogenes,* and *Vibrio* bacteria [38]. The ethanolic and methanolic extracts had the largest mean diameter of non-growth halo against gram--negative *E. coli* at the concentrations studied, with mean diameters of 23.8767 and 16.5067 mm, respectively, extracts from medicinal plants (Apiaceae) are becoming a more important source of new drugs and healthcare items, these results showed *Torilis arvensis* extracts have high effect against gram--negative which agree with our results [39]. Methanol extracts of Apiaceae species have antioxidant, enzyme inhibitory, antibacterial, and cytotoxic activities [40]. A new study attempts to discover novel antimicrobial drugs that have fewer adverse effects and are more effective [41]. Herbs have been used to treat infectious diseases for centuries [42]. Herbal extracts and essential oils have recently been studied for their antimicrobial properties. For hundreds of years, plant extracts (Torilis arvensis) have been utilized as natural treatments to treat a wide range of ailments, including bacteria, fungi, and viruses [43].

Conclusions

Acinetobacter baumannii isolated from burns and wounds showed high resistance against antibiotics. *Acinetobacter baumannii* produces a strong biofilm formation at 55%. Alcoholic extracts (methanol and ethanol) of *Torilis arvensis* at 200 $mg.ml^{-1}$ showed a high effect against MDR isolates of *A. baumannii*.

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Study of Antibacterial Activity from Alcoholic Extracts of *Torilis arvensis* (Huds.) Link on Biofilm formation of *Acinetobacter baumannii* in Diyala Province - Iraq

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