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Original article

Antibacterial activity of an aqueous extracts of *Alkanna tinctoria* roots against drug resistant aerobic pathogenic bacteria isolated from patients with burns infections

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Abstract: *Objective* — *Alkanna tinctoria* (*A. tinctoria*) which is one of the most important medical plants worldwide, has been reported to have antimicrobial activities against some gram-positive and gram-negative bacteria.
Material and Methods — To investigate the ability of four different concentrations of cold-water and hot-water extracts of *A. tinctoria* roots to inhibit growth of 395 isolates of drug resistance bacteria isolated from patients with burns infections during the period of June 2015 to June 2016 in Iraq. Evaluation of *A.tinctoria* roots aqueous extract antibacterial activity (cold and boiling water) was done by use of agar well diffusion method.
Results — The results proved that out of 395 total bacterial isolates there were 321 (81.3%), 37 (9.4%) and 11 (2.8%) isolates with multi-drug resistance (MDR), extensive drug resistance (XDR) and pan-drug resistance (PDR), respectively. Also, the results revealed that 300 mg/ml of the hot-water extract was the best effective inhibitor of the growth, it's inhibited growth of all MDR, XDR and PDR bacteria, the inhibition zone diameter for *Pseudomonas aeruginosa* (132 strains), *Klebsiella pneumoniae* (99 strains), methicillin sensitive *Staphylococcus aureus* (72 strains), methicillin resistant *Staphylococcus aureus* (41 strains), *Escherichia coli* (32 strains), *Acinetobacter baumannii* (11 strains) and *Proteus* ssp. (8 strains) were 28.44±0.11 mm, 28.46±0.25 mm, 28.53±0.24 mm, 28.37±0.12 mm, 27.88±0.09 mm, 28.001±0.001mm, and 27.59±0.23 mm, respectively. All inhibition zones diameter were not significantly different (P>0.05) from that for imipenem 10 mg, chosen as positive control.
Conclusion — From the overall results obtained it is evident that the hot-water extract (300 mg/ml) of *A. tinctoria* roots has excellent anti-bacterial activity against bacteria associated with burns infections. *A. tinctoria* roots may be considered as a raw material for the manufacture of ointment for treatment of burns infections.

Keywords: aqueous extracts, *Alkanna tinctoria*, multi-drug resistance, bacteria, burns infections

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Introduction

Many microbial infections caused by multi-drug resistant (MDR) bacteria, extensive drug resistant (XDR) and pan-drug resistant (PDR) such as *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E.coli*) and methicillin resistant *Staphylococcus aureus* (MRSA) are closely related with prolonged hospitalization and mortality rates due to the limited antimicrobial therapeutic options for infected patients [1, 2].

In recent years, control of infections acquired in communities and hospitals caused by MDR, XDR and PDR bacteria has become a major problem in both developing and developed countries [3, 4]. Drug resistant gram-negative and gram-positive bacteria produce enzymes that bestow resistance to most beta-lactam antibiotics such as Amoxicillin, Amoxclave, Cefotaxime, Ceftriaxone and Ceftazidime [5].

Plants are important source for antibacterial compounds. These compounds can be obtained from different parts of the plant such as roots, leaves and flowers. Many herbal medicines derived from medical plant extracts are being used in the treatment of many bacterial infections such as burns infections. Many researchers have reported that plants contain very important constituents used as antibacterial compounds [6].

Many medical herbs are still being used by both rural and urban communities to treat many bacterial infections such as burns infections. *Alkanna tinctoria* (*A. tinctoria*) which is one of the most important medical plants worldwide, has been reported to have antimicrobial activities against some gram-positive and gram-negative bacteria [7] . However, in Iraq, there is no study on investigation of the antibacterial effects of *A. tinctoria* against drug resistance bacteria. Therefore, in this study, we investigated the antibacterial activity of the extract of *A.tinctoria* roots against multidrug resistance *P. aeruginosa*, *K. pneumoniae*, methicillin sensitive and methicillin resistant *Staphylococcus aureus* (*S. aureus*), *E. coli*, *Acinetobacter baumannii* (*A. baumannii*) and *Proteus* ssp. isolated from patients with burns infections.

Table1. Numbers and percentage of aerobic pathogenic bacteria isolated from patients with burns infections in central hospital of Al-Kufa City (Iraq)

	P. aeruginosa	*MSSA*	*MRSA*	*K. pneumoniae*	*E. coli*	*A. baumannii*	*Proteus ssp.*	*Total*
Single isolate	100 (25.3)	66 (16.7)	31 (7.8)	85 (21.5)	4 (1.0)	6 (1.5)	2 (0.5)	294 (74.4)
Mix isolates	32 (8.1)	6 (1.5)	10 (2.5)	14 (3.5)	28 (7.1)	5 (1.3)	6 (1.5)	101 (25.6)
Total isolates	132 (33.4)	72 (18.2)	41 (10.4)	99 (25.1)	32 (8.1)	11 (2.8)	8 (2.0)	395 (100)

N=395 isolates. Data presented as numbers and percentage (from N) – no. (%). MSSA, methicillin sensitive *S. aureus*; MRSA, methicillin resistant *S. aureus*.

Table 2. Resistance pattern of aerobic pathogenic bacteria isolated from patients with burns infections in central hospital of Al-Kufa City (Iraq)

Antimicrobials	*P. aeruginosa (132 strains)*	*K. pneumoniae (99 strains)*	*MSSA (72 strains)*	*MRSA (41 strains)*	*E. coli (32 strains)*	*A. baumannii (11 strains)*	*Proteus ssp. 8 strains*	*Total (395 strains)*
AMC 30mg	132 (100)	95 (96.0)	63 (87.5)	38 (92.7)	27 (84.4)	8 (72.7)	5 (62.5)	368 (93.2)
CTX 30mg	125 (94.7)	94 (94.9)	61 (84.7)	36 (87.8)	18 (56.3)	7 (63.6)	4 (50.0)	345 (87.3)
CRO 30mg	121 (91.7)	93 (93.9)	52 (72.2)	38 (83.7)	17 (53.1)	7 (63.6)	6 (75.0)	334 (84.6)
CAZ 30mg	119 (90.2)	95 (96.0)	58 (80.6)	37 (90.2)	19 (59.4)	8 (72.7)	6 (75.0)	342 (86.6)
IMP 10mg	25 (18.9)	10 (10.1)	2 (2.8)	7 (17.1)	0 (0)	0 (0)	0 (0)	44 (11.1)
GM 15mg	98 (74.2)	90 (90.9)	53 (72.6)	35 (85.5)	15 (46.9)	6 (54.5)	4 (50.0)	301 (76.2)
TM 10mg	118 (89.4)	89 (89.9)	58 (80.6)	32 (78.0)	15 (46.9)	6 (54.5)	4 (50.0)	322 (81.5)
AN 30mg	110 (83.3)	88 (88.9)	31 (43.1)	32 (78.0)	12 (37.5)	7 (63.6)	3 (37.5)	283 (71.6)
C 30mg	115 (87.1)	81 (81.8)	28 (38.9)	31 (75.6)	14 (43.8)	8 (72.7)	3 (37.5)	280 (70.9)
CIP 5mg	93 (70.5)	69 (69.7)	22 (30.6)	12 (29.3)	10 (31.3)	5 (45.5)	2 (25.0)	213 (53.9)

Data presented as numbers and percentage of aerobic pathogenic bacterial isolates that were resistant to antimicrobials – no. (%).
AMC, amoxicillin with clavulanic acid; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; IMP, imipenem; GM, gentamicin; TM, tobramycin; AN, amikacin; C, chloramphenicol; CIP, ciprofloxacin; MSSR, methicillin sensitive *S. aureus*; MRSA, methicillin resistant *S. aureus*.

Material and Methods

Bacterial isolates

A total of 395 swabs were collected from patients with burns infections in central hospital of Al-Kufa City (Iraq) during the period of June 2015 to June 2016. All swabs were streaked onto the surface of blood agar (Oxoid, UK), MacConkey agar (Oxoid, UK) and mannitol salt agar (Oxoid, UK) and incubated overnight at 37°C. All emergent colonies were identified by standard bacteriological methods (Growth on MacConkey Agar, Gram stain, Capsule stain, Triple Sugar Iron test, Catalase and oxidase test, Indole production test, Methyl Red test, Voges-Proskauer test, Simmons Citrate test and Coagulase test) [8] and then by cultivation on Chrome agar medium (Orientation Company, France). Finally all isolates were identified by using Vitek2 system (BioMerieux, France). Detection of methicillin resistant *S. aureus* strains were done by incubated all *S. aureus* strains overnight at 37°C in 5 ml of Mueller-Hinton broth (Oxoid, UK). All samples were streaked onto the surface of CHROMagar MRSA media (Orientation Company, France) by sterile swabs (Bioanalyse, Turkey) and incubated overnight at 37°C. The growth of more than two colonies will indicate methicillin-resistance [9].

Antimicrobials susceptibility test

Kirby-Bauer method was performed for antibiotic susceptibility testing according to the Clinical Laboratory Standards Institute (CLSI, 2014) [10]. Briefly, fresh bacterial strain with 0.5 McFarland turbidity was swabbed onto the Mueller-Hinton agar (Oxoid, UK) surface using sterile swab sticks. Antimicrobial discs (Bioanalyse, Turkey) were evenly embedded onto the inoculated agar incubated at 37°C overnight. Ten antimicrobials were used in this study: amoxicillin with clavulanic (AMC) acid 20/10 mg, cefotaxime (CTX) 30 mg, ceftriaxone (CRO) 30 mg, ceftazidime (CAZ) 30 mg, imipenem (IMP) 10 mg, gentamicin (GM) 15 mg, tobramycin (TM) 10 mg, amikacin (AN) 30 mg, Chloramphenicol (C) 30 mg, ciprofloxacin (CIP) 5 mg. Antimicrobial susceptibility and resistance was determined according to CLSI guidelines (2014) [10] according to strain growth zone diameter. *E. coli* ATCC 35218 and *S. aureus* ATCC 25923 strains were used as control. According to the results of antimicrobials susceptibility test: any bacterial isolate resist to a minimum at least 3 different classes of antibiotics it is multi-drug resistance (MDR), any bacterial isolate remain susceptible to only one or two class of antibiotics it is extensive-drug resistance (XDR) and any bacterial isolate resistance to all sub classes in all classes of antibiotics it is pan-drug resistance (PDR) [10, 11].

Collection and identification of A. tinctoria and preparation of plant extract

A. tinctoria was obtained from medicinal plant herbarium. It was identified by the Department of Botany, Faculty of Science, University of Kufa (Iraq). *A. tinctoria* roots were freed from foreign particles, washed with water to remove dust, dried and ground to a powder. Fifty gram from roots powder was extracted with 500 ml cold sterile water and boiling water for overnight. The extracted solutions were then filtered through a 0.2 µm membrane filter (Whatman, USA) and evaporated to dryness at 45°C [6]. The extracts were kept sterile containers and stored at 4°C.

Antibacterial activity test

The antibacterial activity of aqueous extract (cold and boiling water) of *A. tinctoria* roots was done according to method by Rauha et al. (2000) [12] and Radji et al (2013) [3] by use of the agar well diffusion method as follow: Five or three fresh bacterial colony with 0.5 McFarland turbidity was swabbed onto the Mueller-Hinton agar surface (Oxoid, UK) using sterile swab (Bioanalyse, Turkey). Three wells (five mm in diameter) were made in each Mueller-Hinton agar (Oxoid, UK) plate by use of a sterile cork borer (Himedia-India). The crude roots of plant extracts were serially diluted to yield dilutions of 50, 100, 200, and 300 mg/ml, and 50 µl of each dilution was transferred to each well and left for three hours at 21–23°C to enable diffusion of the extract across the surface. The plates were then incubated at 37°C for 24 h. The inhibition zone around each well was measured in millimeters. All tests were carried out in triplicates.

Table 3. Resistance type of aerobic pathogenic bacteria isolated from patients with burns infections in central hospital of Al-Kufa City (Iraq)

Resistance type	*P. aeruginosa (132 strains)*	*K. pneumoniae (99 strains)*	*MSSA (72 strains)*	*MRSA (41 strains)*	*E. coli (32 strains)*	*A. baumannii (11 strains)*	*Proteus ssp. (8 strains)*	*Total (395 strains)*
MDR	122 (92.4)	83 (83.8)	58 (80.6)	33 (80.5)	18 (56.3)	5 (45.5)	2 (25.0)	321 (81.3)
XDR	21 (15.9)	11 (11.1)	2 (2.8)	2 (4.9)	1 (3.1)	0 (0)	0 (0)	37 (9.4)
PDR	7 (5.3)	2 (2.0)	0 (0)	2 (4.9)	0 (0)	0 (0)	0 (0)	11 (2.8)

Data presented as numbers and percentage of aerobic pathogenic bacterial isolates that were resistant to antimicrobials – no. (%).
MDR, multi-drug resistance; XDR, extensive drug resistance; PDR, pan-drug resistance; MSSR, methicillin sensitive *S. aureus*; MRSA, methicillin resistant *S. aureus*.

Statistical analysis

Statistical analysis was performed with SPSS version 10 software by use of the t test. P <0.05 was regarded as indicative of statistical significance.

Results

Numbers and percentage of bacterial isolates

From the 395 swabs isolated from patients with burns infections, the most prevalent aerobic pathogenic bacteria was *P. aeruginosa* (132 isolates, 33.4%) while the least was *Proteus* ssp. (8 isolates, 2.0%) (*Table* 1).

Antibiotic susceptibility test

The results of this study proved that *P. aeruginosa*, *K. pneumoniae*, methicillin sensitive and methicillin resistant *S. aureus* were highly resistant to most antimicrobials especially to amoxicillin + clavulanic acid (20/10 mg) and third-generation cephalosporins, while *E.coli*, *A. baumannii* and *Proteus* ssp. were less resistant to these antimicrobials. The results indicated that out of the 395 total bacterial isolates there were 368 isolates (93.2%) were resistant to amoxicillin + clavulanic acid (20/10 mg), while there were only 44 isolates (11.1%) were resistant to imipenem (10 mg) (*Table* 2). The results of this study proved that imipenem (10 mg) was the best of the antibiotics tested. Imipenem (10 mg) was therefore used as positive control to compare with antibacterial activity of aqueous extract of *A. tinctoria* roots against bacterial isolates. On the other hand, the results proved out of the 395 total bacterial isolates there were 321 isolates (81.3%) were multi-drug resistance, 37 isolates (9.4%) were extensive drug resistance and 11 isolates (2.8%) were pan-drug resistance (*Table* 3).

Antibacterial activity of an aqueous extract of A. tinctoria roots

The results of the current study demonstrated that cold-water extracts 50 and 100 mg/ml of *A. tinctoria* roots had no any inhibitory activity against all bacterial isolates, while cold-water extracts 200 and 300 mg/ml, and hot-water extract 50 mg/ml of *A. tinctoria* roots had inhibitory activity against some bacterial isolates resulting in the inhibition zones ranging between 22.61±0.26 to 24.85±0.13 mm of cold-water extracts 200 mg/ml against *P. aeruginosa* and *E.coli*, respectively, 23.87±0.35 to 24.88±0.09 mm of cold-water extracts 300 mg/ml against methicillin resistant *S. aureus* and *K. pneumoniae*, respectively, 24.92±0.46 to 25.08±0.07 mm of hot-water extracts 50 mg/ml against *E.coli* and methicillin sensitive *S. aureus*, respectively.

Also, the results proved that hot-water extracts 100 and 200 mg/ml of *A. tinctoria* roots had inhibitory activity against large number of bacterial isolates resulting in the inhibition zones zones ranging between 24.83±0.30 to 26.09±0.47 mm of hot-water extracts 100 mg/ml against methicillin sensitive *S. aureus* and *E.coli*, respectively, 26.08±0.08 to 26.77±0.23 mm of hot-water extracts 200 mg/ml against methicillin sensitive *S. aureus* and *A. baumannii*, respectively.

The study also demonstrated that hot-water extract 300 mg/ml of *A. tinctoria* roots has inhibitory activity against all bacterial resulting in the largest inhibition zones ranging between 27.59±0.23 to 28.53±0.24 mm against *Proteus* ssp. and methicillin sensitive *S. aureus* (*Table* 4).

The results were indicative of a significant difference P<0.05 between imipenem 10 mg/ml (*Table* 5) and hot-water extracts 50, 100, 200 mg/ml (*Table* 6). On the other hand, the result proved that there was non-significant difference P>0.05 between imipenem 10mg and the hot-water extract 300 mg/ml (*Table* 7).

Hot-water extract 300 mg/ml has the best antibacterial activity and produced large inhibition zones 28.18±0.09 mm, 28.21±0.11 mm and 28.32±0.11 mm against all multi-drug resistance, extensive drug resistance and pan-drug resistance of aerobic pathogenic bacterial isolates, respectively, and there was non-significant difference P>0.05 with imipenem 10mg (*Table* 7).

Discussion

Burns infections are one of the most dangerous problems in hospitals, and MDR gram-positive and gram-negative bacteria are the most dominant etiological agents of this infections include, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *E.coli*, *A. baumannii*, *Proteus* ssp. and others [13-16].

Many classes of antimicrobials were used to prevent this type of infections such as beta-lactam, cephalosporins, aminoglycosides and others, but unfortunately, most pathogenic bacteria cause burns infections became more resistant to these antimicrobials by different means such as the vertical and horizontal transfer of antimicrobial resistance gene and became multi-drug resistance (one bacterial isolate resist to a minimum at least 3 different classes of antibiotics) or extensive-drug resistance (one bacterial isolate remain susceptible to only one or two class of antibiotics) or pan-drug resistance (one bacterial isolate resistance to all sub classes in all classes of antibiotics) [17-19].

Therefore, different medical plants were used by many researchers as alternative treatment against different bacterial infections because these medicinal plants are the rich source of traditional medicines [3, 6, 20-22].

A. tinctoria is one of the best medical plants used as plant herbal therapy for treating different infections worldwide [3, 23]. In Iraq there is no studies focused on the antibacterial activity of *A. tinctoria*, therefore, the present study was designed to evaluation the antibacterial activity of cold-water and hot-water extracts of *A. tinctoria* roots against drug resistance aerobic pathogenic bacteria isolated from patients with burns infections.

Table 4. Evaluation of the antibacterial activity of cold-water and hot-water extracts of *A. tinctoria* roots against aerobic pathogenic bacteria isolated from patients with burns infections in central hospital of Al-Kufa City (Iraq)

Aerobic pathogenic bacteria	*Concentration, no. (%), M±SE mm*			
	50 mg/ml	*100 mg/ml*	*200 mg/ml*	*300 mg/ml*
Cold-water extracts, R=3				
P. aeruginosa (132 strains)	0 (0), Resistance	0 (0), Resistance	2 (1.5), 22.61±0.26	8 (6.1), 24.48±0.26
K. pneumoniae (99 strains)	0 (0), Resistance	0 (0), Resistance	3 (3.0), 23.42±0.30	8 (8.1), 24.88±0.09
MSSA (72 strains)	0 (0), Resistance	0 (0), Resistance	2 (2.8), 23.57±0.25	6 (8.3), 25.16±0.11
MRSA (41 strains)	0 (0), Resistance	0 (0), Resistance	3 (7.3), 23.49±0.26	3 (7.3), 23.87±0.35
E. coli (32 strains)	0 (0), Resistance	0 (0), Resistance	2 (6.3), 24.85±0.13	4 (12.5), 24.69±0.34
A. baumannii (11 strains)	0 (0), Resistance	0 (0), Resistance	1 (9.1), 23.32±0.29	1 (9.1), 24.05±0.05
Proteus ssp. (8 strains)	0 (0), Resistance	0 (0), Resistance	1 (12.5), 23.43±0.28	1 (12.5), 24.40±0.30
Hot-water extracts, R=3				
P. aeruginosa (132 strains)	16 (12.1), 24.67±0.28	60 (45.5), 24.86±0.14	100 (75.8), 26.14±0.07	132 (100), 28.44±0.11
K. pneumoniae (99 strains)	18 (18.2), 24.54±0.24	50 (50.5), 24.90±0.11	90 (90.9), 26.43±0.16	99 (100), 28.46±0.25
MSSA (72 strains)	17 (23.6), 24.51±.28	25 (34.7), 24.83±0.30	66 (91.7), 26.08±0.08	72 (100), 28.53±0.24
MRSA (41 strains)	15 (36.6), 25.08±0.07	25 (61.0), 24.93±0.20	35 (58.4), 26.45±0.29	41 (100), 28.37±0.12
E. coli (32 strains)	8 (25.0), 24.92±0.46	21 (65.6), 26.09±0.47	32 (100), 26.85±0.15	32 (100), 27.88±0.09
A. baumannii (11 strains)	3 (27.3), 24.61±0.24	5 (45.5), 25.06±0.08	9 (81.8), 26.77± 0.23	11 (100), 28.00±0.001
Proteus ssp. (8 strains)	3 (37.5), 24.21±0.21	6 (75.0), 25.07±0.09	8 (100), 26.37±0.31	8 (100), 27.59±0.23

Data presented as numbers and percentage of aerobic pathogenic bacterial isolates that were sensitive to aqueous extract of *A. tinctoria* roots – no. (%).
R, number of replicates; M, mean of diameter of inhibition zone (mm); SE, standard error of mean; MSSR, methicillin sensitive *S. aureus*; MRSA, methicillin resistant *S. aureus*.

Table 5. Comparison between imipenem 10mg (positive control) and cold-water extracts of *A. tinctoria* roots (200 and 300 mg/ml) against aerobic pathogenic bacteria isolated from patients with burns infections in central hospital of Al-Kufa City (Iraq)

Aerobic pathogenic bacteria	*Cold-water extracts, no. (%), M±SE mm*		*Imipenem 10mg, R=3, no. (%), M±SE mm*	*P-value A*	*P-value B*
	200 mg/ml, R=3	*300 mg/ml, R=3*			
P. aeruginosa (132 strains)	2 (1.5), 22.61±0.26	8 (6.1), 24.5±0.26	107 (81.1), 28.78±0.46	<0.001	<0.001
K. pneumoniae (99 strains)	3 (3.0), 23.42±0.30	8 (8.1), 24.88±0.09	88 (88.9), 28.47±0.27	<0.001	<0.001
MSSA (72 strains)	2 (2.8), 23.57±0.25	6 (8.3), 25.16±0.11	70 (97.2), 28.33±0.33	<0.001	<0.001
MRSA (41 strains)	3 (7.3), 23.49±0.26	3 (7.3), 23.87±0.35	34 (82.9), 28.47±0.26	<0.001	<0.001
E. coli (32 strains)	2 (6.3), 24.85±0.13	4 (12.5), 24.69±0.34	32 (100) 28.29±0.30	<0.001	0.001
A. baumannii (11 strains)	1 (9.1), 23.32±0.29	1 (9.1), 24.05±0.05	11 (100), 28.13±0.08	<0.001	<0.001
Proteus ssp. (8 strains)	1 (12.5), 23.43±0.28	1 (12.5), 24.40±0.30	8 (100), 28.16±0.43	<0.001	0.002

Data presented as numbers and percentage of aerobic pathogenic bacterial isolates that were sensitive to imipenem 10mg and aqueous extract of *A. tinctoria* roots – no. (%). A – P-value of imipenem 10mg and cold-water extract 200 mg/ml; B – P-value of imipenem 10mg and cold-water extract 300 mg/ml.
R, number of replicates; M, mean of diameter of inhibition zone (mm); SE, standard error of mean. MSSR, methicillin sensitive *S. aureus*; MRSA, methicillin resistant *S. aureus*.

The results of the present study proved that *P. aeruginosa*, *K. pneumoniae* and *S. areus* were the most dominant pathogenic bacteria isolated from patients with burns infections (*Table* 1), also the result demonstrated that most bacterial isolates were highly resistant to most antimicrobials (*Table* 2) and out of total 395 isolates there were 321 isolates (81.3%) were MDR (*Table* 3).

In recent years, large numbers of gram-negative and gram-positive bacteria become increasingly important pathogens in both community settings and hospitals, such as *P. aeruginosa*, *K. pneumoniae*, *S. areus* and others. These pathogenic bacteria play an important role in the colonization and infection of patients with burns infections [24, 25].

Treatment of those patients is often very difficult due to cross-resistance of drug-resistant bacteria with a large group of antimicrobials. Therefore, we need new sources of natural compounds with antibacterial activity against drug-resistance pathogenic bacteria.

This study aimed to evaluate the ability of different concentrations (50, 100, 200 and 300 mg/ml) of cold-water and hot-water extracts of *A. tinctoria* roots to inhibit growth of 395 drug resistance pathogenic bacteria isolated from patients with burns infections.

The results demonstrated that both cold-water 200 and 300 mg/ml and hot-water extracts 50, 100, 200 and 300 mg/ml of *A. tinctoria* roots had different inhibitory activity against bacterial isolates with the hot water extract at 300 mg/ml resulting in the largest inhibition zones (*Table* 4).

The results were indicative of a significant difference P<0.05 between imipenem 10mg (positive control) and the cold-water extracts 200 and 300 mg/ml (*Table* 5) and all hot-water extracts at different concentrations (*Table* 6). Also, the hot-water extract (300 mg/ml) inhibited all MDR, XDR and PDR bacterial isolates that were resistant to imipenem 10mg (*Table* 7).

This is an indication that hot water is a better than cold water as solvent for extracting the anti-bacterial compounds in *A. tinctoria* roots. These findings were indicative of the presence of active antibacterial compounds in this plant.

A. tinctoria is herbal medicines with antibacterial activity has been proved to possess health promotion properties and medicinal compounds, including the ability to inhibit the growth of gram-positive and gram-negative pathogenic bacteria and cause wound healing [7, 26, 27].

Table 6. Comparison between imipenem 10mg (positive control) and hot-water extracts of *A. tinctoria* roots (50, 100, 200, and 300 mg/ml) against aerobic pathogenic bacteria isolated from patients with burns infections in central hospital of Al-Kufa City (Iraq)

Aerobic pathogenic bacteria	*Hot-water extracts, no. (%), M±SE mm*				*Imipenem 10mg, R=3, no. (%), M±SE mm*	*P-value*			
	50 mg/ml, R=3	*100 mg/ml, R=3*	*200 mg/ml, R=3*	*300 mg/ml, R=3*		*A*	*B*	*C*	*D*
P. aeruginosa (132 strains)	16 (12.1), 24.67±0.28	60 (45.5), 24.86±0.14	100 (75.8), 26.14±0.07	132 (100), 28.44±0.11	107 (81.1), 28.78±0.46	0.002	0.001	0.005	0.509
K. pneumoniae (99 strains)	18 (18.2), 24.54±0.24	50 (50.5), 24.90±0.11	90 (90.9), 26.43±0.16	99 (100), 28.46±0.25	88 (88.9), 28.47±0.27	<0.001	<0.001	0.003	0.971
MSSA (72 strains)	17 (23.6), 24.51±0.28	25 (34.7), 24.83±0.30	66 (91.7), 26.08±0.08	72 (100), 28.53±0.24	70 (97.2), 28.33±0.33	<0.001	0.001	0.003	0.652
MRSA (41 strains)	15 (36.6), 25.08±0.07	25 (61.0), 24.93±0.20	35 (58.4), 26.45±0.29	41 (100), 28.37±0.12	34 (82.9), 28.47±0.26	<0.001	<0.001	0.007	0.751
E. coli (32 strains)	8 (25.0), 24.92±0.46	21 (65.6), 26.09±0.47	32 (100), 26.85±0.15	32 (100), 27.88±0.09	32 (100), 28.29±0.30	0.004	0.013	0.013	0.263
A. baumannii (11 strains)	3 (27.3), 24.61±0.24	5 (45.5), 25.06±0.08	9 (81.8), 26.77±0.23	11 (100), 28.00±0.01	11 (100), 28.13±0.08	<0.001	<0.001	0.005	0.190
Proteus ssp. (8 strains)	3 (37.5), 24.21±0.21	6 (75.0), 25.07±0.09	8 (100), 26.37±0.31	8 (100), 27.59±0.23	8 (100) 28.16±0.43	0.001	0.002	0.028	0.299

Data presented as numbers and percentage of aerobic pathogenic bacterial isolates that were sensitive to imipenem 10mg and aqueous extract of *A. tinctoria* roots – no. (%). A – P-value of imipenem 10mg and hot-water extract 50 mg/ml; B – P-value of imipenem 10mg and hot-water extract 100 mg/ml; C – P-value of imipenem 10mg and hot-water extract 200 mg/ml; D – P-value of imipenem 10mg and hot-water extract 300 mg/ml.
R, number of replicates; M, mean of diameter of inhibition zone (mm); SE, standard error of mean. MSSR, methicillin sensitive *S. aureus*; MRSA, methicillin resistant *S. aureus*.

Table 7. Comparison between imipenem 10mg (positive control) and hot-water extract of *A. tinctoria* roots 300 mg/ml against multi-drug resistance, extensive drug resistance and pan-drug resistance of aerobic pathogenic bacteria isolated from patients with burns infections in central hospital of Al-Kufa City (Iraq)

Resistance type	*Total, no. (%)*	*Imipenem 10mg, R=3, no. (%), M±SE mm*	*Hot-water extract 300 mg/ml, R=3, no. (%), M±SE mm*	*P-value*
MDR	321 (81.26)	290 (90.3), 28.18±0.09	321 (100), 28.18±0.09	0.953
XDR	37 (9.36)	31 (83.8), 28.19±0.10	37 (100), 28.21±0.11	0.917
PDR	11 (2.78)	0 (0), Resistant	11 (100), 28.32±0.11	ND

Data presented as numbers and percentage of aerobic pathogenic bacterial isolates that were sensitive to imipenem 10mg and hot-water extract of *A. tinctoria* roots – no. (%). R, number of replicates; M, mean of diameter of inhibition zone (mm); SE, standard error of mean; MDR, multi-drug resistance; XDR, extensive drug resistance; PDR, pan-drug resistance; ND, not done.

A. tinctoria root contain pharmaceutical compounds with a wide spectrum of biological properties such as hydroxynaphthoquinones (HNQ) and isohexenylnaphthazarins (IHN) are potent pharmaceutical substances with a well-established and wide spectrum of antibacterial, wound healing, anticancer and anti-inflammatory activities, in this study, the wide spectrum of anti-bacterial effect of *A. tinctoria* root extracts may be due to alkannin esters and shikonin semi-quinone radical formation (naphthoquinone), exhibiting cytotoxicity via the generation of endogenous superoxide anion radicals [28-32].

The results demonstrated that hot-water extract and a high concentration (300 mg/ml) of an active principle in the extracts of *A. tinctoria*; alkannin esters and shikonin might be responsible for this anti-bacterial activity.

Conclusion

The present study proved that hot-water extracted from *A. tinctoria* roots 300 mg/ml has very good antibacterial activity against all drug-resistance bacteria isolated from patients with burns infections. So, *A. tinctoria* roots may be considered as a raw material for the manufacture of ointment for treatment of burns infections.

Conflict of interest

We declare that we have no conflict of interest.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

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