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In vitro antibacterial activity of an aqueous extracts of *Matricaria chmomilla* flowers against pathogenic bacteria isolated from pregnant women with urinary tract infection.

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Abstract

Objective: *Matricaria chmomilla* is one of the most important medical plants use in both developed and developing countries to treat many infections. The main aim of this study was to investigate the ability of an aqueous extracts of *Matricaria chmomilla* flowers to inhibit growth of pathogenic bacteria isolated from pregnant women with urinary tract infection.

Methods: This is a descriptive cross-sectional study that was carried out at the laboratory of microbiology in Al-Najaf central hospital in Al-Najaf Governorate, Iraq during March to December 2017. A total of 600 urine samples were collected from 600 pregnant women (age range 25-35 y old) infected with urinary tract infection. Kirby-Bauer method was performed for antibiotic susceptibility testing. Agar well diffusion method was used for the antibacterial activity of cold and boiling water extracts of *M. chmomilla* flowers in three concentrations (50, 100 and 150) mg/ml.

Results: Out of 600 urine samples, 654 bacterial strains were isolated. *Escherichia coli* was the most predominant bacteria (278 isolates, 42.5%) followed by *Klebsiella pneumoniae* (201 isolates, 30.7%), *Acinetobacter baumannii* (112 isolates, 17.1%), *Enterobacter aerogenes* (23 isolates, 3.5%), *Citrobacter freundii* (18 isolates, 2.8%), *Proteus mirabilis* (12 isolates, 1.8%) and *Staphylococcus saprophyticus* (10 isolates, 1.6%). Imipenem 10 µg was the best antimicrobial against all bacterial isolates. Hot water extract (150 mg/ml) has excellent anti-bacterial activity against all bacterial isolates, the inhibition zone diameters of *E. coli*, *K. pneumoniae*, *A. baumannii*, *E. aerogenes*, *C. freundii*, *P. mirabilis* and *S. saprophyticus* were 29.3 ± 0.2, 26.3 ± 0.4, 26.3 ± 0.2, 28.3 ± 0.3, 29.3 ± 0.1, 29.3 ± 0.5 and 28.3 ± 0.2, respectively and there was non-significant differences (P-value>0.05) between imipenem 10 µg (positive control) and 150 mg/ml of hot-water extracts.

Conclusion: *Matricaria chmomilla* flowers may be considered as a raw material for the manufacture of new drug for treatment of urinary tract infection in human.

Keywords: *Matricaria chmomilla*, Aqueous extracts, Antibacterial, Urinary tract infection.

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Introduction

Medical plants are one of the most important mean to treat of many bacterial infections due to it has different antibacterial compounds and don't have side effects against human [1]. *Matricaria chmomilla* (*M. chmomilla*) also called *Matricaria recutita* commonly known as German Chamomile or Chamomile belong to family Asteraceae and is widely used as antibacterial in the worldwide such as in Europe, Africa and Asia [2]. Before 1000 y ago, this medical plant was used in Egypt, Greece and Rome to treat different infections and it is considered as one of nine sacred herbs [3,4]. *Matricaria chmomilla* has been used treat different illness such as gastrointestinal infection, cold and diarrhoea [5,6]. *Matricaria chmomilla* flowers are one of the most important parts of this plant has been used in many medical treatments like antipyretic and carminative [7]. Urinary Tract Infection (UTI) is one of the most recurrent infections infect individuals especially in women worldwide [8]. *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and other enterobacteriaceae family are the common cause of UTI [9]. The emergence of multi-drug resistant pathogenic bacteria such as *E. coli*, *K. pneumoniae*, *Acinetobacter baumannii* (*A. baumannii*) and *Staphylococcus saprophyticus* (*S. saprophyticus*) that cause UTI and other different infections led to worsens problem [10-12]. Therefore, these drugs must be replaced with another antibacterial compounds have potent effect against multi-drug resistant pathogens and without side effects against individuals [13]. Urinary tract infection is common in pregnant women [14]. This infection may be cause dangerous complications in fetus during pregnancy; on the other hand, pregnant women infected with UTI cannot intake of antibiotics because of the dangerous effect on the foetus that may be lead to death or distortions after birth. Therefore this study was aimed to

evaluation of antibacterial effect of hot and cold-water extracts of *M. chmomilla* flowers against pathogenic bacteria isolated from pregnant women with urinary tract infection as alternative medical compounds against these pathogens.

Materials and Methods

Study design

This is a descriptive cross-sectional study that was carried out at the Laboratory of Microbiology in Al-Najaf Central Hospital in Al-Najaf Governorate, Iraq during March to December 2017. 600 urine samples were collected from 600 pregnant women (age rang 25-35 y old) infected with urinary tract infection.

Urine culture, identification of bacterial isolates and antibiotics susceptibility test

All urine samples were streaked immediately onto blood agar (Oxoid, UK) surface and Macconkey agar (Oxoid, UK) surface by sterile loop (Hi-media, India) and incubated aerobically at 37°C for 24 h. Any growth of bacterial isolates was in titer $\leq 10^5$ colony forming units were considered as a positive growth. All bacterial isolates were identified according to standard bacteriological methods [15]. Antibiotics susceptibility testing was performed by a disc diffusion test according to Kirby-Bauer method [16]. Resistance and sensitive of bacterial isolates to antibiotics were determined according to CLSI guidelines [17].

Collection, identification and preparation of an aqueous extracts of M. chmomilla

Matricaria chmomilla was collected from herbal market of Al-Najaf Governorate- Iraq and it was identified by University of Kufa, Faculty of Science, and Department of Botany. *Matricaria chmomilla* flowers were collected and freed from foreign particles and washed with water to remove dust, dried and ground to powder. 50 g from flowers were extracted with 500 ml cold sterile water and boiling water for 24 h. The extracted solutions were then filtered through a 0.2 µm membrane filter (Whatman, USA) and evaporated at 40°C [18]. The extracts were kept in sterile containers (Hi-media-India) at 3°C until use.

Antibacterial activity test

Antibacterial activity testing of cold-water and hot- water extracts was done by use the agar well diffusion method [19] briefly, 5 to 3 fresh bacterial colonies with 0.5 MacFarland turbidity was swabbed onto the Mueller-Hinton agar (Oxoid, UK) agar using sterile swab (Hi-media, India). Three wells with 5 mm in diameter were made in each Mueller-Hinton agar (Oxoid, UK) surface by sterile cork-borer (Hi-media, India). The crud flowers of *M. chmomilla* extracts were serially diluted to yield dilutions of 50, 100, and 150 mg/ml. 50 µl of each dilution was transfer to each well and left for 3 h at 20°C to enable diffusion of the extract across agar surface and incubated at 37°C overnight aerobically. All tests were carried out in triplicates. The inhibition zone around each well was measured in millimetres.

Statistical analysis

Unpaired T-test was performed in statistical analysis by use SPSS-10 windows software. If P value<0.05 was regarded as indicative of statistical significance. Data were presented in numbers (No) and percentages (%) and mean ± standard error of mean.

Results

Of the 600 urine samples were collected from pregnant women, 654 were positive for bacterial growth. *E. coli* was the most predominant bacteria 278 (42.5%) followed by *K. pneumoniae* 201 (30.7%), *A. baumannii* 112 (17.1%), *E. aerogenes* 23 (3.5%), *C. freundii* 18 (2.8%), *P. mirabilis* 12 (1.8%) and *S. saprophyticus* 10 (1.6%) (Table 1).

Among 12 antimicrobials were used in susceptibility pattern, all isolates were highly resistant to most antimicrobials, imipenem was the best antimicrobial showed the full activity (100%) against most bacterial isolates, therefore it chosen as positive control. The antimicrobials susceptibility pattern of all bacterial isolates is shown in Table 2 and Figure 1.

As shown in Table 3, Hot water extract (150 mg/ml) has excellent anti-bacterial activity against all bacterial isolates, the inhibition zone diameters of *E. coli*, *K. pneumoniae*, *A. baumannii*, *E. aerogenes*, *C. freundii*, *P. mirabilis* and *S. saprophyticus* were 29.3 ± 0.2, 26.3 ± 0.4, 26.3 ± 0.2, 28.3 ± 0.3, 29.3 ± 0.1, 29.3 ± 0.5 and 28.3 ± 0.2, respectively and there was non-significant differences (P-value>0.05) between imipenem 10 µg (positive control) and 150 mg/ml of hot-water extracts as shown in Table 4.

Table 1. *Numbers and percentage of pathogenic bacteria isolated from pregnant women with urinary tract infection.*

	E. coli	*K. pneumoniae*	*A. baumannii*	*E. aerogenes*	*C. freundii*	*P. mirabilis*	*S. saprophyticus*	**Total**
Single isolate	256 (42.6)	178 (29.7)	107 (17.8)	19 (3.2)	18 (3)	12 (2)	10 (1.7)	600 (91.7)
Mix isolates	22 (40.7)	23 (42.6)	5 (9.3)	4 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	54 (8.3)

Total	278 (42.5)	201 (30.7)	112 (17.2)	23 (3.5)	18 (2.8)	12 (1.8)	10 (1.5)	654 (100)

Data presented as numbers and percentage (from N)-no (%).

Table 2. *Resistance pattern of pathogenic bacterial isolates from pregnant women with urinary tract infection.*

	E. coli **(N=278)**	*K. pneumonia* **(N=178)**	*A. baumannii* **(N=107)**	*E. aerogenes* **(N=19)**	*C. freundii* **(N=18)**	*P. mirabilis* **(N=12)**	*S. saprophyticus* **(N=10)**	**Total (N=654)**
AMC 30 µg	200 (71.9)	170 (95.5)	100 (93.4)	12 (63.1)	14 (77.7)	10 (83.3)	9 (90)	515 (78.7)
CRO 30 µg	195 (70.1)	172 (96.6)	100 (93.4)	12 (63.1)	11 (61.1)	9 (75)	8 (80)	507 (77.5)
CTX 30 µg	205 (73.7)	170 (95.5)	99 (92.5)	12 (63.1)	10 (55.5)	9 (75)	7 (70)	512 (78.2)
CAZ 30 µg	185 (66.5)	177 (99.4)	98 (91.5)	11 (57.8)	10 (55.5)	9 (75)	7 (70)	497 (75.9)
GM 15 µg	170 (61.1)	165 (92.6)	99 (92.5)	8 (42.1)	8 (44.4)	7 (58.3)	6 (60)	463 (70.7)
TM 10 µg	174 (62.5)	160 (89.9)	85 (79.4)	9 (47.3)	8 (44.4)	6 (50)	5 (50)	447 (68.3)
NA 30 µg	120 (43.1)	155 (87)	80 (74.7)	9 (47.3)	7 (38.8)	6 (50)	5 (50)	382 (58.4)
C 30 µg	166 (59.7)	168 (94.3)	91 (85)	10 (52.6)	8 (44.4)	8 (66.6)	7 (70)	458 (70)
CIP 5 µg	167 (60)	166 (93.2)	95 (88.7)	9 (47.3)	9 (50)	7 (58.3)	8 (80)	461 (70.4)
LVX 5 µg	188 (67.6)	172 (96.6)	100 (93.4)	11 (57.8)	10 (55.5)	10 (83.3)	9 (90)	500 (76.4)
F 30 µg	190 (68.3)	161 (90.4)	100 (93.4)	12 (63.1)	12 (66.6)	10 (83.3)	9 (90)	494 (75.5)
IMP 10 µg	0 (0.0)	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.3)

Data presented as numbers and percentages of pathogenic bacterial isolates that were resistant to antimicrobials-no (%); AMC: Amoxicilin with clavulanic acid; CRO: Ceftriaxone; CTX: Cefotaxime; CAZ: Ceftazidime; GM: Gentamicin; TM: Tobramycin; NA: Amikacin; C: Chloramphenicol; CIP: Ciprofloxacin; LVX: Levofloxacin; F: Nitrofurantoin; IMP: Imipenem.

Table 3. *Evaluation of the aqueous extracts of Matricaria chmomilla flowers against pathogenic bacteria isolated from pregnant women with urinary tract infection.*

Pathogenic bacteria	**Cold-water extracts, R=3**		
	Concentration, no. (%), M ± SE mm		
	50 mg/ml	**100 mg/ml**	**150 mg/ml**
E. coli (N= 278)	2 (0.7), Resistance	10 (3.5), 18.3 ± 0.2	25 (8.9), 18.3 ± 0.6
K. pneumoniae (N=178)	0 (0), Resistance	0 (0), Resistance	0 (0), Resistance
A. baumannii (N=107)	0 (0), Resistance	0 (0), Resistance	0 (0), Resistance
E. aerogenes (N=19)	0 (0), Resistance	1 (5.2), 18.3 ± 0.3	1 (5.2), 18.4 ± 0.2
C. freundii (N=18)	0 (0), Resistance	1 (5.5), 19.3 ± 0.1	1 (5.5), 19.8 ± 0.2
P. mirabilis (N=12)	0 (0), Resistance	1 (8.3), 15.3 ± 0.5	1 (8.3), 19.3 ± 0.5
S. saprophyticus (N=10)	0 (0), Resistance	0 (0), Resistance	0 (0), Resistance
Pathogenic bacteria	**Hot-water extracts, R=3**		
	Concentration, no. (%), M ± SE mm		
	50 mg/ml	**100 mg/ml**	**150 mg/ml**
E. coli (N= 278)	87 (31.2), 21.5 ± 0.2	278 (100), 24.4 ± 0.2	278 (100), 29.3 ± 0.2
K. pneumoniae (N=178)	66 (37), 18.3 ± 0.2	115 (64.6), 20.3 ± 0.6	178 (100), 26.3 ± 0.4
A. baumannii (N=107)	54 (50.4), 19.3 ± 0.7	98 (91.5), 21.3 ± 0.1	107 (100),26.3 ± 0.2
E. aerogenes (N=19)	15 (78.9), 20.3 ± 0.3	19 (100), 20.2 ± 0.3	19 (100), 28.3 ± 0.3

C. freundii (N=18)	12 (66.6), 20.2 ± 0.1	18 (100), 20.3 ± 0.1	18 (100), 29.3 ± 0.1
P. mirabilis (N=12)	8 (66.6), 21.3 ± 0.5	12 (100), 22.3 ± 0.5	12 (100), 29.3 ± 0.5
S. saprophyticus (N=10)	7 (70), 17.5 ± 0.2	10 (100), 18.3 ± 0.2	10 (100), 28.3 ± 0.2

Data presented as numbers and percentages of pathogenic bacteria that were sensitive to aqueous extracts of *Matricaria chmomilla* flowers-no (%); R: Numbers of replicates; M: Mean of diameter of inhibition zone (mm); SE: Standard Error of mean.

Table 4. *Comparison in antibacterial activity between Imipenem 10 µg (positive control) and hot-water extract 150 mg/ml of Matricaria chmomilla flowers against pathogenic bacteria isolated from pregnant women with urinary tract infection.*

Pathogenic bacteria	Hot-water extracts, 150mg/ml, R=3, no. (%), M ± SE mm.	Imipenem 10 µg, R=3, no. (%), M ± SE mm. (positive control)	P-value
E. coli (N=278)	278 (100), 29.3 ± 0.2	278 (100), 28.9 ± 0.3	0.171
K. pneumoniae (N=178)	178 (100), 26.3 ± 0.4	2 (1.1), 27.1 ± 0.3	0.196
A. baumannii (N=107)	107 (100), 26.3 ± 0.2	107 (100), 26.5 ± 0.5	0.14
E. aerogenes (N=19)	19 (100), 28.3 ± 0.3	19 (100), 27.9 ± 0.5	0.573
C. freundii (N=18)	18 (100), 29.3 ± 0.1	18 (100), 30.1 ± 0.4	0.714
P. mirabilis (N=12)	12 (100), 29.3 ± 0.5	12 (100), 28.8 ± 0.1	0.236
S. saprophyticus (N=10)	10 (100), 28.3 ± 0.2	10 (100), 27.8 ± 0.2	0.264

Data presented as numbers and percentages of pathogenic bacterial isolates that were sensitive to Imipenem 10 µg and 150 mg/ml of hot-water extracts of *Matricaria chmomilla* flowers-no (%); R: Numbers of replicates; M: Mean of diameter of inhibition zone (mm); SE: Standard Error of mean.

Discussion

Matricaria chmomilla is one of the best medical plants were used to treat different infections before 1000 y ago in different countries [20]. According to the best of our knowledge, this is the first study in Iraq aimed to evaluate the antibacterial activity of cold-water and boiling-water extracts of *Matricaria chmomilla* flowers against pathogenic bacteria isolated from pregnant women with UTI. In this study, *E. coli* was the most common bacteria isolated from urine of pregnant women with UTI followed by *K. pneumoniae* and *A. baumannii* as shown in Table 1. Gram-negative bacteria belong to enterobacteriaceae family is one of the most etiological agents of UTI with percentage in-between 80% to 90% [21,22]. While, gram-positive bacteria comes in the second degree, for example, *S. saprophyticus* is the second most common cause UTI in females with percentage between 30% to 40% [23]. Most gram-negative bacteria as well as some of gram-positive bacteria have different virulence factors such as fimbriae, biofilms and capsule that enabling these pathogens to attachment, persistence and colonization in the bladder epithelium of urinary tract and cause infection [24,25]. Our findings showed that most bacterial isolates were resistant to most antibiotics with high percentages as shown in Table 2 and Figure 1, these results are similar with many previous studies in different countries such as, in Egypt, Uganda, India and Nigeria [26-29]. Overuse of different classes of antibiotics such as; aminoglycosides, beta-lactams, 3rd and 4th generation cephalosporin's and quinolones to treat this infection, lead to emerging of new highly antibiotics resistant bacterial strains due to vertical and horizontal transfer of antibiotics resistant gene between different bacterial isolates in the same family or to different family [30-33]. Therefore, many medical plants were used to treat different infections as an alternative medical compounds against highly drug resistant pathogens because of it is contain rich source of traditional medicines [1,18]. In this study, we aimed to evaluate the antibacterial effect of three concentrations (50, 100 and 150) mg/ml of cold-water and boiling-water extracts of *M. chmomilla* flowers to inhibit growth of pathogenic bacteria isolated from urine of pregnant women with UTI, the results showed that 150mg/ml of boiling-water extract had excellent effect against all bacterial isolates with inhibition percentage 100%, the inhibition zone diameters of *E. coli*, *K. pneumoniae*, *A. baumannii*, *E. aerogenes*, *C. freundii*, *P. mirabilis* and *S. saprophyticus* were 29.3 ± 0.2, 26.3 ± 0.4, 26.3 ± 0.2, 28.3 ± 0.3, 29.3 ± 0.1, 29.3 ± 0.5 and 28.3 ± 0.2, respectively .while other concentrations of cold-water and boiling-water extracts don't provided full antibacterial activity against all bacterial isolates as shown in Table 3, and there was non-significant differences (P-value>0.05) in antibacterial effect between imipenem 10 µg (positive control) and 150 mg/ml of hot-water extracts (Table 4). *Matricaria chamomilla* is an important medicinal plant was used in treatment of many infections such as respiratory tract infections and gastrointestinal tract infections in many countries [34]. *Matricaria chamomilla* flowers was used as antipyretic and carminative, in addition, flowers oil has been used in colic, flatulence and rheumatism [35]. One of the most abundant components of the essential flowers oil, apart from it is spasmolytic effects on intestinal smooth muscle [36,37]. Also, has been reported to have antibacterial, antipyretic, antifungal and anti-inflammatory as well as ulcer protective effect [38-40]. *Matricaria chamomilla* which have been associated with relieved hypertensive symptoms and decreases the systolic blood pressure, increasing urinary output and may be lead to decrease the numbers of pathogenic bacteria in urinary tract [41]. Also, drinking *M. chamomilla* was associated with increase in urinary levels of hippurate and glucine, which have been associated with increased antibacterial activity [42]. In the present study, boiling-water

extract had a significant antibacterial activity as compare with cold-water extract; this may be due to that the boiling water has the ability to extracted more medical compounds from flowers more than cold water.

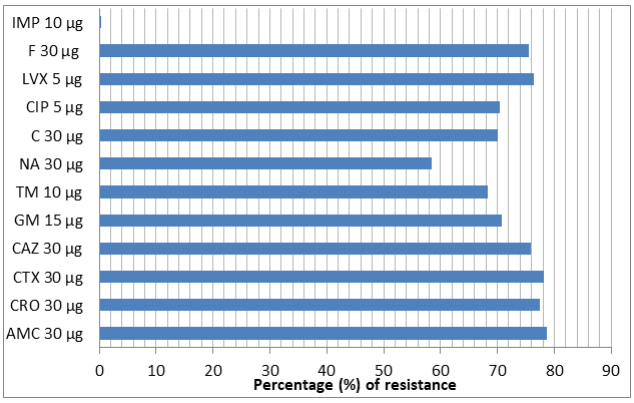

Figure 1. *Overall resistance to 12 antimicrobials of 654 bacterial isolates from pregnant women with urinary tract infection. AMC: Amoxicilin with clavulanic acid; CRO: Ceftriaxone; CTX: Cefotaxime; CAZ: Ceftazidime; GM: Gentamicin; TM: Tobramycin; NA: Amikacin; C: Chloramphenicol; CIP: Ciprofloxacin; LVX: Levofloxacin; F: Nitrofurantoin; IMP: Imipenem.*

Conclusion

This study showed that boiling-water extract (150 mg/ml) of *M. chmomilla* flowers extract has excellent antibacterial activity against all bacterial isolates from pregnant women with UTI. So, *M. chmomilla* flowers may be considered as a raw material for the manufacture of new drug for treatment of urinary tract infection.

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