



Antibacterial Effects of Cranberry and Bearberry Herbs Extracts against *Klebsiella pneumoniae* isolated from UTIs in Baghdad City, Iraq

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Abstract: Urinary tract infections (UTIs) are the second most common type of bacterial infection worldwide. UTIs are gender-specific diseases, with a higher incidence in women. This type of infection could occur in the upper part of the urogenital tract or in the lower part of the urinary tract. The most common etiological agent *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Conventional therapeutic treatment involves the use of antimicrobial agents, but due to the dramatic increase in antimicrobial resistance (AMR), the natural alternatives for UTI treatment represents a current research topic. To compare the antibacterial effects of Cranberry, Bearberry and meropenem against isolated *K. pneumoniae*. Firstly, VITEK2 system was used for identification of bacterial isolates. Then antibacterial activity of Cranberry, Bearberry and meropenem against *K. pneumoniae* were assessed by Minimal inhibitory concentration (MIC) using the broth micro dilution method. All isolated *K. pneumoniae* were obsolete resistance against Ampicillin in 75(100%) and high resistance rate to Cefazolin 65(86%), Ceftriaxone, Cefotaxime, Cefepime, and Trimethoprim/ Sulfamethoxazole 45 (60%). But they showed a low level of resistance rate to Nitrofurantoin, Cefoxitin, Gentamicin 35 (46.6%), 25 (33.3%), and 15 (20%) respectively. While displayed high sensitivity to Ertapenem, Imipenem, Piperacillin/ Tazobactam, Amikacin, Tigecycline at 75(100%). The results of Meropenem MIC were 32mg/ml for the isolated (K1, K3, K4, K6, K7, K8, and K9) strains while the isolate K2, K5 and K10 showed (64mg/ml, 64mg/ml and 16mg/ml) respectively as listed in (table 2 and figure 1). By the other hand, the results showed that the minimum inhibitory concentration of cranberry extract inhibition for *K. pneumoniae* isolate, which were 16mg/ml for isolates (K1, K3, K4, K5, K6, and K7). While 32mg/ml for isolates (K2, K8, K9) and 64mg/ml in K10 (table 2 and figure2). In contrast, the inhibitory effects of bearberry revealed an excellent finding at 16mg/ml for (K1, K3, K4, K5, and K6) while the MICs concentration for K2, K8, K9 and K10 were (32mg/ml, 32mg/ml, 32mg/ml and 64mg/ml) respectively. It can be concluded that the Cranberry and Bearberry possess an obvious antibacterial activity against isolated *K. pneumoniae* in comparison to meropenem.

Keywords: *Klebsiella pneumoniae*, Cranberry, Bearberry, Carbapenemase.

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Introduction

Urinary tract infections (UTIs) are among the most common and serious bacterial infections worldwide. Although they are treatable, controlling

UTIs is becoming increasingly difficult due to the widespread antimicrobial resistance observed in uropathogens, particularly those from the Enterobacteriaceae family (1). The

length of hospital stay and the performance of invasive procedures are risk factors for acquiring *Klebsiella pneumoniae*, *K. pneumoniae* a gram-negative, non-motile, rod-shaped bacterium with a prominent polysaccharide-based capsule (2). *Klebsiella* organisms are resistant to multiple antibiotics, which is believed to be a plasmid-mediated property (3). In healthy people, *K. pneumoniae* is frequently found in the respiratory, gastrointestinal, and urinary systems. Hospitals are linked to the majority of *K. pneumoniae* infections, which can be lethal if improperly treated (4). At least 60% of women will at some point in their lives suffer the symptoms of a urinary tract infection (UTI). In the United States, 10% of women experience one or more symptomatic UTIs year. Young women between the ages of 18 and 24 who engage in sexual activity had the greatest prevalence of UTIs (5). *K. pneumoniae* can leading threat in modern healthcare, recognized as a primary cause of both hospital- and community-acquired infections. Its role in the dissemination of antibiotic resistance genes from environmental bacteria to pathogenic strains cannot be overstated. This dangerous pathogen is responsible for a range of serious infections, including hospital-acquired *pneumoniae*, urinary tract infections, bacteremia, surgical site infections, ventilator-associated *pneumoniae*, and septicemia. Moreover, it poses a grave risk to immunocompromised patients, leading to opportunistic infections that can be life-threatening. Combating *K. pneumoniae* is essential in our fight against antibiotic resistance and ensuring patient safety (6). Blueberries (*Vaccinium* spp.) have a wide range of health advantages and nutritional

components (7). Blueberries are known for their excellent antioxidant properties, which are attributed to their high total anthocyanin content (8). Anthocyanin varieties contain different levels of glycosides, providing colors to flowers, fruits, and other organs of plants (9). Anthocyanin also has great potential benefits to human health due to its strong oxidation resistance for enhancing immunity capacity and balancing weight (7, 10). Sun investigated the inhibition effects of anthocyanins found in wild blueberries on the growth of *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Vibrio parahaemolyticus* (11). Industrially-used methods for extracting anthocyanins from natural sources are commonly based on conventional solvent extraction (12). Owing to the unique qualities of bioactive compounds, such as high adsorption ability and convenient desorption, absorption separation with macroporous resins is an effective technique for isolating anthocyanins from plant crude extracts (3).

Materials and Methods

Bacterial isolate

In this study, a total of 150 samples as clinical specimens (UTI infection), from both sexes (male and female) and from different age groups that admitted to Al-Yarmouk General Hospital and Al-Karama General Hospital in Baghdad City, Iraq. This study was conducted between August 2023 to November 2024. All urine samples were positioned in clean sterile containers. Urine samples were firstly cultured on blood agar, and MacConkey agar media, and then incubated for 24 h at 37 °C.

Plants

Cranberry and Bearberry that used in the current study were purchased from VITEK2 system

The isolates were identified using the Vitek 2 automated system (Vitek 2 GN-card), which employs fluorescence-based technology. This system includes IDGNB cards, Vitek 2 software, and Advanced Expert System (AES) software from bioMérieux, used per the manufacturer's instructions. Bacterial strains were sub-cultured onto MacConkey agar plates to ensure purity. Suspensions were adjusted to a McFarland standard of 0.5 in sterile sodium chloride solution, with preparation and card filling completed in under 30 minutes. The VITEK 2 IDGNB card, containing 64 wells and 41 fluorescent biochemical tests, was used for identifying gram-negative bacilli. After sealing and incubating the cards for 3 hours, they were monitored every 15 minutes using kinetic fluorescence measurements. The VITEK 2 software analyzed the data and automatically reported the results. This procedure was done according to Khafagy *et al.*, (14).

MIC of Meropenem and Bearberry

Minimal inhibitory concentration (MIC) estimation using the broth microdilution method (15). The antibiotics used in this method were meropenem, Cranberry and Bearberry.

Statistical Analysis

Statistical analysis was done using SPSS version 26 to determine the percentage value.

Results and Discussion**Antibiotic Susceptibility profile of *K. pneumoniae* Isolates from Humans:**

Most of isolated *K. pneumoniae* have been resistant to a wide range variety of examined antibiotics. All isolated *K. pneumoniae* were obsolete resistance rate against Ampicillin in 75(100%) and the results revealed a high resistance rate to Cefazolin 65(86%), Ceftriaxone, Ceftazidime, Cefepime,

Trimethoprim/Sulfamethoxazole 45 (60%). But they showed a low level of resistance rate to Nitrofurantoin, Cefoxitin, Gentamicin 35 (46.6%), 25 (33.3%), and 15 (20%) respectively. While the bacterial isolated revealed high sensitivity to Ertapenem, Imipenem, Piperacillin/ Tazobactam, Amikacin, Tigecycline at 75(100%). These results were shown (Table 4-3). The resistant rate of *K. pneumoniae* against all tested revealed a highly significantly different with a p-value < 0.001. These supported results through observation conducted by Al-Salihi *et al.* (16) who founded that *K. pneumoniae* were more resistant to ampicillin (100%). As well as Nirwati *et al.*, (17) who found a good result compared in terms of hypersensitivity to Piperacillin/Tazobactam, Imipenem, and Amikacin. The main causes of *K. pneumoniae* resistance to antibiotics including beta-lactam antibiotics are the production of a wide range of beta-lactamase which is one of the most important problems of increasing infection in hospitals, or by changing the permeability barrier or at the target site represented by the penicillin binding protein, or a change in the protein of the outer membrane (18).

Table (1): Antibiotic sensitivity profile of 150 sample of *K. pneumoniae* isolated from UTIs.

Classes	Members	Number and % Resistance rate	Number and % Sensitive rate
B-Lactam	Ampicillin	150(100%)	0
	Piperacillin/ Tazobactam	0	150(100%)
	Cefazolin	130(86.6%)	20(13.3%)
	Ceftriaxone	90(60%)	60(40%)
Cephems	Cefoxitin	50(33.3%)	100(66.6%)
	Ceftazidime	90(60%)	60(40%)
	Cefepime	90(60%)	60(40%)
Carbapenems	Ertapenem	0	150(100%)
	Imipenem	0	150(100%)
Aminoglycosides	Amikacin	0	150(100%)
	Gentamicin	30(20%)	120(80%)
Fluor quinolones	Ciprofloxacin	10(6.6%)	140(93.3%)
	Levofloxacin	10(6.6%)	140(93.3%)
Glycylcycline	Tigecycline	0	150(100%)
Nitro furans	Nitrofurantion	70(46.6%)	10(6.6%)
Sulfonamides	Trimethoprim/ Sulfamethoxazole	90(60%)	60(40%)
X2	585.79		
P value	0.001*		

X2: chi-square test, * significant difference (P<0.05).

Minimum inhibitory concentration

In the current study, ten isolates of *Klebsiella pneumoniae* have been chosen which were multi-drug resistant. The lowest concentrate of an antimicrobial drug that would inhibit observable microorganism growth after a period of incubation is termed a minimum inhibitory concentration (MIC). Clinical laboratories utilize MICs primarily to confirm resistance, however, they are also used as a research tool to determine the activity of new antimicrobial agents as well as their MIC breakpoints (19). The MIC of the cranberry, bearberry and meropenem antibiotic were determined using the microtiter plate method on 96-well. An oxidation-reduction colorimetric indicator is used in this procedure. The MIC of antimicrobial drugs against *K. pneumoniae* was measured by resazurin stain. Resazurin salt dye, which is blue when oxidized,

turns pink when reduced by live cells, and the MIC can be determined without the use of a spectrophotometer (20). The results of Meropenem MIC were 32mg/ml for the isolated (K1, K3, K4, K6, K7, K8, and K9) strains while the isolate K2, K5 and K10 showed (64mg/ml, 64mg/ml and 16mg/ml) respectively as listed in table 2 and Figure (1). By the other hand, the results showed that the minimum inhibitory concentration of cranberry extract inhibition for *K. pneumoniae* isolate, which were 16mg/ml for isolates (K1, K3, K4, K5, K6, and K7). While 32mg/ml for isolates (K2, K8, K9) and 64mg/ml in K10 (table 2 and figure2). In contrast, the inhibitory effects of bearberry revealed an excellent finding at 16mg/ml for (K1, K3, K4, K5, and K6) while the MICs concentration for K2, K8, K9 and K10 were (32mg/ml, 32mg/ml, 32mg/ml and

64mg/ml) respectively as described in (table 2 and figure 3).

The results of the study involved determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Cranberry and Bearberry extracts on the studied bacteria, *K. pneumoniae*. The findings showed that Bearberry extract had a higher inhibitory effect against the tested bacteria. The results indicate that *K. pneumoniae* demonstrated greater sensitivity to Bearberry extract and Cranberry. Our results are consistent with the findings of the authors findings who reported the greater susceptibility of *K. pneumoniae* to Cranberry extract (21). Furthermore, the agreement between our results and the results of Rashid, (22) provides further evidence supporting of the antibacterial effects of Bearberry extract against *K. pneumoniae* (22). Previous studies have also confirmed the antibacterial properties of Cranberry extracts. Baenas and Ruales (23) identified Cranberry extract as a natural antioxidant agent, possessing in vitro and in vivo antioxidant capacity (23). Amin Salehi,

(24) reported that the phenolic compounds in Cranberry fruit extracts have been suggested to influence bacterial cell growth. These compounds decrease the permeability of bacterial cell walls, resulting in disturbances in ion transport. Furthermore, Guevara-Terán (25) found that polyphenols are the predominant compounds in berries, serving as the main bioactive compounds with antioxidant capacity and inhibitory effects against Gram-positive and Gram-negative bacteria; which aligns with our findings (25). Previous reports have also indicated that the impact of Cranberry extract on inhibiting bacterial growth varies depending on the developmental stage of the fruit. Extensive research has demonstrated the antimicrobial properties of many berry extracts, attributing their inhibitory effect to the high content of phenolic constituents (26, 27). However, most of the efforts were focused on investigating the antimicrobial effect against foodborne- and urinary tract infection related pathogens (28).

Table (2): MIC of Cranberry, Bearberry and Meropenem on *Klebsiella* isolates.

Isolate	MIC of Meropenem (mg/ml)	MIC of Cranberry (mg/ml)	MIC of Bearberry (mg/ml)
<i>k</i> ₁	32	16	64
<i>k</i> ₂	64	32	64
<i>k</i> ₃	32	16	64
<i>k</i> ₄	32	16	64
<i>k</i> ₅	64	16	64
<i>k</i> ₆	32	16	64
<i>k</i> ₇	32	16	64
<i>k</i> ₈	32	32	64
<i>k</i> ₉	32	32	64
<i>k</i> ₁₀	16	64	64

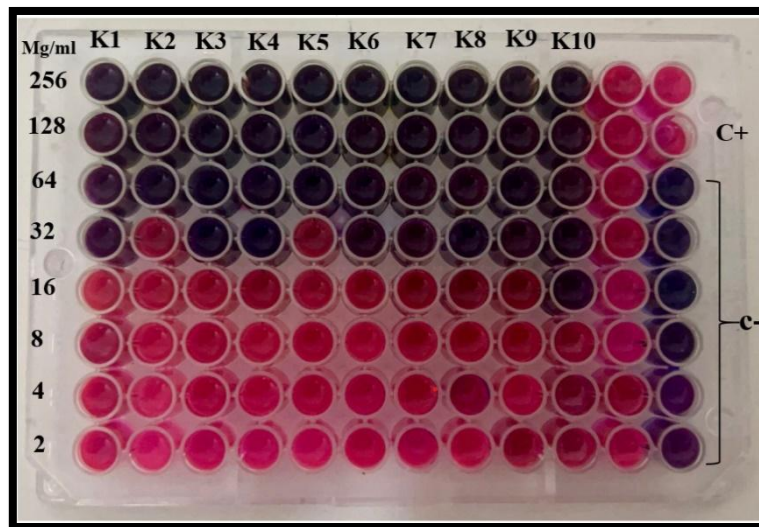


Figure (1): MIC of Meropenem.

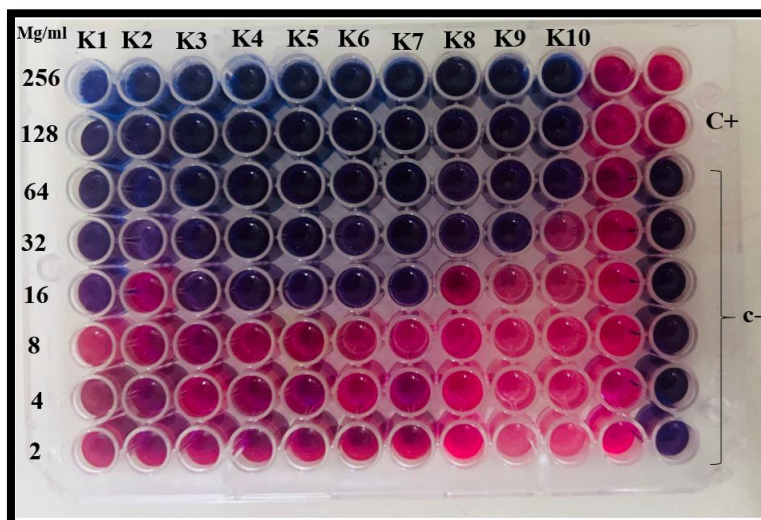


Figure (2): MIC of Cranberry.

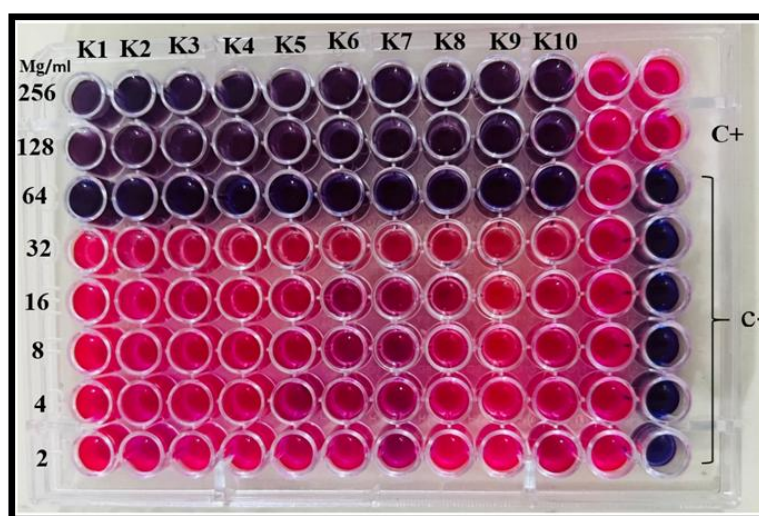


Figure (3): MIC of Bearberry.

Conclusion

In conclusion, Cranberry and Bearberry possess an obvious antibacterial activity against *K. pneumoniae* isolated from UTI in comparison to meropenem. And these blue and black berries may be a potential candidates for antibacterial applications. However, further clinical trials are needed to validate the current findings.

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