

Antimicrobial Activity of Grape Seed Extracts on Some Species of Bacteria that Cause Urinary Tract Infection

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Abstract: The study included the collection of (50) samples of diuresis from patients suffering from Urinary tract infection (UTI) in Al-Kindi Teaching Hospital in Baghdad and used sterile bottles for sample collection and cultured immediately after taking the sample for diagnosis. Bacterial isolates were diagnosed by performing cultured and biochemical tests and the results showed that *Escherichia coli* is the most common species in (UTI) patients and by (15) 47% isolation of the total 32 isolates, about (10) 31.5% from *K. pneumonia*, and (7) 21.5% isolates of *P.aerugenosa*. The sensitivity of the bacteria to (5) antibiotics (Amikacin, Ciprofloxacin, Erythromycin, Vancomycin and Gentamycin) were investigated and among different groups, all of which are sensitive to Amikacin and Ciprofloxacin, all of which are resistant to Erythromycin, *E.coli* and *K. pneumonia*, sensitive to Vancomycin, Gentamycin while *P. aerugenosa* resistant to them. The inhibitory effect of aqueous and alcoholic extracts of grape seed was studied for four concentrations (25), (50), (100), (200) mg/ml, for the studied bacteria. The extracts showed inhibitory effectiveness and varying effects depending on the type, concentrations of extract, and the type of microorganism. The inhibitory capacity and the effectiveness ratio of aqueous extracts was weak compared to alcoholic extracts.

Key points: Urinary tract infection, *Escherichia coli*, grape seed extract, Sensitivity test.

Introduction:

The urinary tract infection is a major health problem and is one of the most susceptible areas to bacterial infection and the highest incidence of infection with *E. coli*, secondly *Proteus spp.*, then *Klebsiella spp.* and *Staphylococcus* and finally *Enterobacter spp.* (Sonavane *et al.*, 2008 ; Li *et al.*, 2022). It affects all ages and females are more affected than males and the reason is due to the indiscriminate use of antibiotics and leads to resistance of new strains to antibiotics and becomes less efficient for the treatment of urinary tract inflammation (Tilgher, 2000 ; Alasmay, 2021). Antimicrobial resistance is emerging as a major worldwide concern. Up until recently, β -lactam antibiotics made up more than 50% of all antibiotics sold commercially (Yahya Abdulla *et al.*, 2022). Eighty to eighty-five percent of UTIs are caused by gram-negative bacteria, with *Escherichia coli* accounting for fifty to seventy percent of prevalent causes, followed by *Klebsiella* (Sarojamma & Ramakrishna, 2011), *Enterobacteriaceae*, of which *E. coli* is a part, are the most common causative bacteria associated with UTIs, accounting for as many as 80% of cases (Shakya *et al.*, 2017). Over the last twenty years, antibiotics have been extensively employed in the treatment of pathogenic microbes. Nevertheless, the abuse of these medications has led to the global emergence of antimicrobial resistance, a critical health concern that has emerged in recent years (Chiang *et al.*, 2018), and because of the lack of new antibiotics, treating multiple illnesses is becoming more difficult as antibiotic resistance rises throughout the world. Antimicrobial resistance (AMR) infections claim the lives of about 700,000 people worldwide each year, and by 2050, that number is predicted to increase to 10 million (Jia *et al.*, 2021).

Therefore, researchers were interested in herbs and the use of plant extracts and consider them alternatives to medicines, a large number of them have come up with treatments of medicinal herbs, that plants differ in terms of active substances and their chemical qualities (El-Mahrouk, 2016 ; Al-Rawi *et al.*, 2012). Many plant extracts have been tested and developed, WASTES OF PRESSED GRAPES, *Olea europea* extract, *trigonella foenum* extract, and others, to study their effectiveness against pathogens and use them as a safe alternative to antibiotics (Al-Rawi *et al.*, 2012 ; Al-Shuwaikh *et al.*, 2019 ; Ibrahim, 2020) One of the fruits that is most often consumed worldwide is grapes. The leaves and sap of grape bushes have long been utilized in traditional European medicine. In addition to being a great source of vitamins and fiber, grape skin and seeds are also incredibly rich in polyphenols, notably proanthocyanidins, which may be utilized as a functional component to improve the body's natural bioprocesses and treat a variety of health conditions and Numerous studies have demonstrated the advantages of proanthocyanidin-rich grape seed extract in the treatment of a wide range of illnesses, including microbial infections, cancer, hypertension, inflammation, diabetes, and ulcerative colitis. Therefore, in addition to being employed as a cosmeceutical or nutraceutical, they may be able to complement or replace already prescribed medications in the treatment of ailments by incorporating it into other effective pharmaceutical formulations for improved prospects down the road (Gupta *et al.*, 2020) most recent studies have tended to study the effectiveness and benefits of grape seeds extract (GSE) because of its effective effect against many types of bacteria and contains active substances including phenol and is called Broad spectrum grape (Al-Jumaily *et al.*, 2011 ; Al-Rawi *et al.*, 2012 ; Krasteva *et al.*, 2023).

MATERIALS AND METHODS

50 samples of diuresis were collected after from patients at Al-Kindi Teaching Hospital in Baghdad suffering from urinary tract infection and were used for the purpose of collecting sterile bottles and the intermediate diuretics sample was taken cultured immediately after the sample was taken for the purpose of diagnosis.

Isolation and diagnosis of bacterial isolates

It was cultured on the agricultural media, including MacConkey agar, nutrition agar and blood agar, and the petri-dish were incubated at a temperature of 37 ° C and for 24 hours for the purpose of initial isolation of bacteria, and took one pure colony and initially diagnosed for the morphological qualities of the size, shape, color and texture of the colonies and then performed purification operations to study their cultivating qualities and bacterial isolates were diagnosed based on bacteriological and biochemical tests (Levinson, 2016).

Antibiotic isolates sensitivity test

- a. Preparation of the culturing medium: Prepare the medium of Mueller Hinton agar, pour in sterile petri-dishes and leave to harden at room temperature, put the dishes in the incubator at a temperature of 37 °C and for 24 hours to make sure they are free of contamination.
- b. Preparation of bacterial suspension: The bacterial suspension was prepared and compared with a standard turbidity constant solution (McFarland) that gives 1.5×10^8 cells/ml. By adding a quantity of saline physiological solution and bacteria to the value corresponding to the reading of the tube containing the standard solution (Soussy, 2001).

c. Preparation of plant extracts

Collection of plant samples

Grape seeds were thoroughly washed with water to remove all dust and then dried naturally at laboratory temperature, and the grape seeds were ground with an electric grinder and kept until use (Hashem, 2009).

Methods of plant extraction

Grape seeds were extracted by the aqueous extraction method based on the Al-Salami method (1998). The plant was also extracted alcoholically using 95% ethyl alcohol and in both methods we

concentrated the solutions extracted by a differential rotary evaporator device and then put them in the dried oven until the extract dries, and then took 1 g of alcoholic or aqueous extract and dissolved with 10% ml of distilled water or DMSO solution to obtain a concentration of 100 mg/ml which is considered a Stock solution to prepare different concentrations of 20, 50, 100, 200 mg/ml.

Qualitative chemical detection of some effective groups of plant extracts

- a. Detection of alkaloids
- b. Detection of tannins
- c. Detection of glycosides
- d. Detection of resins
- e. Detection of saponins
- f. Detection of coumarin
- g. Detection of flavonoids
- h. Detection of steroid and terpene (Hashem, 2009).

Effect of plant extracts on the growth of bacteria isolated from cases of urinary tract infection

The well diffusion assay was used to determine the effectiveness of these aqueous and alcoholic extracts against isolated bacteria and the bacterial suspension was prepared for each isolation and compared it with a McFarland standard solution of which was spread 0.1 ml on the medium of Muller-Hinton agar. Well were made on the cultured medium using sterile cork pour, which is 5 mm in diameter, and added to each hole 0.05 ml of extracts, then a well was made in the center of each petri-dish and added to it 0.05 of DMSO solution 10%, which is used to dissolve alcoholic plant extracts, and was considered a control group, then incubated the petri-dishes at a temperature of 37 °C and for a period of 24 hours and then measured the inhibition diameter around each well by the ruler graduated (Kharma *et al.*, 2006).

Antibiotic sensitivity test for bacterial isolates

The medium of Mueller-Hinton agar was prepared and then its pH was adjusted. The medium was pour into sterile petri-dishes and leave to harden at room temperature, the petri-dishes are placed in the incubator at 37°C and for 24 hours to make sure they are free of contamination and the bacterial stranded was prepared and compared with the standard turbidity constant McFarland which gives 1.5×10^8 cell/ml (Soussy, 2001).

The test was carried out by transferring 0.1 ml of bacterial suspension and spreading on the surface of the petri-dishes and distributed with petri-dishes homogeneously, and the petri-dishes were left to dry at room temperature for 15-20 minutes and then antibiotic discs were placed in the petri-dishes and transferred with forceps by 5 discs per petri-dish and incubated the petri-dishes at a temperature of 37°C for 24 hours, after which the results were read by measuring the diameters of the inhibition zone around the discs and compared with what was stated in CLSI (2019).

Result and discussion

After collecting diuresis samples, detecting the presence of bacteria by culturing, studying the culturing characteristics and biochemical tests as in Table 1. 15 isolates of *E. coli* bacteria were isolated by 47% of the total isolates 32 isolates and about 10 isolates from *K. pneumonia* bacteria by 31.5%. These bacteria are the second cause of urinary tract infection and also cause wounds and burns (Coudroun and Molland, 2002). Hazardous most urinary tract infections, including pyelonephritis and cystitis, are caused by *Escherichia coli*, which is more prevalent and common in women than in men (Kumar Shrestha *et al.*, 2022). There are several reasons why women experience UTIs more frequently than males, including the anatomy of the female, the female urethra is significantly shorter than the male one, and the mucosa—the moist tissue lining the inside of the vagina—makes up the majority of the more sensitive skin of the external urethral meatus in

women, and this skin is more fragile and sensitive than the majority of the skin on the body); the female urethra is situated closer to the abdominal cavity; spermicide or a diaphragm used for birth control, menopause, and pregnancy can cause vaginal frustration; and sexual contact can allow bacteria near the vagina to enter the urethra, making women more vulnerable to infection (August & De Rosa, 2012). One significant human pathogen that causes infections in the urinary tract, pneumonia, bloodstream infections, bacteremia, and sepsis is *Klebsiella pneumoniae*, there are now far fewer therapies for *K. pneumoniae* infections due to the emergence of multidrug-resistant variants. However, there is an urgent need for better management techniques due to *K. pneumoniae* activity and accompanying illnesses (Barani *et al.*, 2022). The current study's findings are mostly in line with another that found that *K. pneumoniae* (11.4%) and *E. coli* (66%) were the two most common bacteria in UTI (Ahmad *et al.*, 2009), *E. coli* and *K. pneumoniae* were found to be prevalent in Isfahan, Iran, at 50.1% and 23.3%, respectively, whereas Kattel *et al.* (2008) stated that *K. pneumoniae* was found to be prevalent in Nepal at 10.78% and *E. coli* was found to be 59.59% (Kattel *et al.*, 2008). The majority of the research concurred that the uropathogens, which comprised *E. coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, and maybe *Candida sp.*, should be arranged from most frequent to least common (Jaggi *et al.*, 2012 ; Jalil & Al Atbee, 2022) *K. pneumoniae* colonies on MacConkey agar medium are large and have a mucous and pink texture because they ferment lactose sugar (Suhartono *et al.*, 2023). There were seven isolates out of a total of 32 isolates (21.5%) are for *P.aeruginosa* bacteria, *P. aeruginosa* urinary tract infections are linked to elevated rates of morbidity and death in senior hospital patients (Gomila *et al.*, 2018). The World Health Organization (WHO) has designated *P. aeruginosa* as an antibiotic-resistant microbe of the utmost concern. The most prevalent UTI bacteria, *Escherichia coli*, usually has lower levels of antibiotic resistance than these isolates (Ironmonger *et al.*, 2015), and previous research has demonstrated that *P. aeruginosa* may infiltrate lung epithelial cells, ocular cells, and most recently, urinary epithelial cells (Kroken *et al.*, 2018 ; Penaranda *et al.*, 2021) these bacteria produce Pyocyanin and Fluorescein pigments in their cultured medium and hemolytic the blood on the blood agar medium and do not produce the enzyme Haemolysin and do not glycolysis the sugars (Azemin *et al.*, 2022 ; Kassob & Hummadi, 2023).

Antibiotic isolates sensitivity test

The susceptibility of bacteria to antibiotics was tested by the method of discs after isolating and diagnosing the bacteria causing urinary tract infections to see the extent of resistance or sensitivity (Table 1). In the medical field, quickly determining the antibiotic susceptibility of microorganisms found in biofluids is a difficult but essential laboratory task. The diagnosis of a urinary tract infection (UTI), for example, is a multistep procedure that takes 24 to 48 hours from pathogen identification to the so-called antibiogram. Before a final diagnosis can be made, repeated culture and the administration of broad-spectrum antibiotics may be necessary (Dina *et al.*, 2023). The capacity of bacteria to endure and remain alive when exposed to antimicrobial drugs is known as antimicrobial resistance (AMR). Antibiotics, disinfectants, and food preservatives are a few examples of antimicrobial agents that may be employed against microorganisms to lessen their growth potential, prevent their reproduction, or even kill them. A variety of natural, semi-synthetic, and synthetic agents with unique mechanisms can significantly alter physiological and metabolic processes. Examples of these agents include β -lactams and glycopeptides, which modify the synthesis of cell walls; macrolides and tetracyclines, which inhibit protein synthesis; sulfonamides, which block metabolic pathways; and fluoroquinolones, which interfere with the replication and translation of DNA (Zhou *et al.*, 2015). In this study namely, Amikacin (Am) has a sensitivity to all isolated bacteria and the Ciprofloxacin antibiotic showed sensitivity to all types of bacteria to the bacteria *E. coli*, *K.pneumoniae*, and *P. aeruginosa*.

The erythromycin (E) antibiotic showed resistance to all species; *E. coli*, *K.pneumoniae* and *P. aeruginosa*. The result was somewhat consistent with Adnan *et al.* (2014) and identical to Hashem (2009) as well as the antibiotic Vancomycin (VA) was used and showed that the bacteria *K.pneumoniae* and *P. aeruginosa* was resistant to this antibiotic and *E. coli* bacteria were sensitive to this antibiotic. The gentamycin (Gn) antibiotic showed sensitivity to both species *E. coli* and

K.pneumoniae and resistance to the *P. aeruginosa* and this result was inconsistent with Astal, (2005). According to our research, erythromycin is a poor antibiotic for treating urinary tract infections. Concerningly high gentamicin and erythromycin resistance rates. Comparable information was gleaned from an investigation carried out in Iraq by Al-Nashbandi and colleagues (Al-Naqshbandi *et al.*, 2019).

The most common infection are urinary tract infections (UTIs), which include a wide range of etiologic agents, a high frequency of occurrences, relapses, and consequences. One major difficulty facing medical professionals is drug resistance in the pathogenic bacteria (Joya *et al.*, 2022), therefore, there have been many researches and studies, including the current study, to test the therapeutic effectiveness of many plant extracts for use as alternative treatments in treating multiple diseases that affect humans, including urinary tract infections (Yossef, 2009 ; Al Taae *et al.*, 2012 ; AL-taie, 2014 ; Majeed, 2017).

Inhibitory effectiveness of aqueous and alcoholic extracts of grape seeds

The results showed that the alcoholic and aqueous extract of the seeds of the grape plant has varying effects on the growth of bacterial isolates *K.pneumoniae*, *E.coli* and *P. aeruginosa* and this depends on the type of microorganism and the concentration used. The results also showed that the inhibition capacity of aqueous extracts was weak compared to the alcoholic extract where it gave high effectiveness to inhibit bacteria and most of them concentrates 50,100 mg/ml.

Whereas the aqueous and alcoholic ones did not show any activity against bacterial growth at a concentration of 25 mg/ml, and showed weak effectiveness for both extracts at a concentration of 50 mg/ml with an inhibition zone of 2 mm for *E.coli*, 0 mm for *K.pneumoniae* and *P. aeruginosa* for the aqueous extract (50 mg/ml), while 6 mm against *P. aeruginosa*, 10 mm against *E.coli*, and 12 mm against *K.pneumoniae* in relation to the alcoholic extract (50 mg/ml), while the extracts showed good antibacterial activity against the growth of the bacteria under study at a concentration of 100 mg/ml, the zone of inhibition is 6 mm for *P. aeruginosa*, 8 mm for *E.coli*, and 12 mm for *K.pneumoniae* with respect to the aqueous extract (100 mg/ml). On other hand 10 mm against *P. aeruginosa* and 17 mm against *E.coli* and 19 mm for *K.pneumoniae* relative to the alcoholic extract (100 mg/ml). While a concentration of 200 mg/ml of the aqueous and alcoholic extract showed excellent antibacterial activity, as the bacterial growth inhibition zone was 10 mm for *Pseudomonas*, 20 mm for *E*, and 22 mm for *K.pneumoniae* for the aqueous extract (200 mg/ml), and 20 mm for *P. aeruginosa*, 23 mm for *E.coli*, and 28 mm for *K.pneumoniae* for the alcoholic extract (200 mg/ml).

According to Basavar *et al.* (2022), grape seeds contain polyphenolic hesperidine, naringin compound and meoheperidine and these substances have high antioxidant effectiveness and their ability to break down the biofilm of bacteria and lead to the leakage of cytoplasmic contents and this process takes place within (10) minutes and in general the increase in the concentration of the alcoholic extract led to an increase in the diameters of bacterial inhibition.

Many experimental investigations have been conducted, confirming the extract's positive health effects. Research has demonstrated that a grape seed extract high in proanthocyanidins can help prevent and treat a wide range of illnesses, including microbial infections, cancer, diabetes, peptic ulcers, cardiovascular disease, and hypertension. Thus, in addition to its potential use as a nutraceutical or cosmeceutical, it may also be developed into additional effective pharmaceutical formulations for improved future prospects, which might serve as a supplement to or replacement for presently used pharmaceuticals in the treatment of ailments (Gupta *et al.*, 2020).

Study on using plant extracts as substitute antioxidants and antibacterial agents, particularly grape seed extracts, are continually expanding (Han *et al.*, 2021 ; Awad *et al.*, 2022). grapes, particularly their seeds and skins, are rich in polyphenols that possess strong antioxidant, antibacterial, and anti-lipid properties (Di Stefano *et al.*, 2022), and the majority of polyphenols (60–70%) are found in grape seeds (Memar *et al.*, 2019). The connection between the antibacterial action of GSEs and their polyphenolic components is not well understood (Pfukwa *et al.*, 2019), the antimicrobial

properties of pure phenolic compounds have been studied by several authors. According to their findings, the degree of polymerization, the quantity of hydroxyl groups, and the chemical structure of phenolic compounds all affect how antimicrobial they are. (Chávez *et al.*, 2006). According to Khameneh *et al.* (2019), phenolic compounds exhibited distinct modes of action in relation to various strains of bacteria, Phenolic chemicals included in grape seed extract may work through a variety of processes to stop bacteria from developing a complete resistance. Pure phenolic compounds have not demonstrated the same antibacterial efficacy as phenolic extracts (Silva *et al.*, 2021), This might be because the extracts' constituent parts work in concert with one another (Ghafoor *et al.*, 2020)

The effectiveness of the alcoholic extract was more inhibitory than that of aqueous extract because it contained glycoside compounds, resins, saponins, phenols and coumarin (Kerekes *et al.*, 2019). The best concentrations were 200 mg/ml for the alcoholic extract and its inhibitory diameters were 23, 28, 20, and for the aqueous extract 20, 22, 10. Thus, the alcoholic extract is better than aqueous as shown in Table 3.

Conclusions

Based on the results of the current study, grape seed extract has an antibacterial activity against bacterial growth, and the alcoholic extract is more effective than the aqueous extract, Antibacterial effectiveness increases with increasing concentration and according to the species of bacteria. Hence, the need for more comprehensive future studies appears to determine the effectiveness of these extracts as an alternative or supportive treatment for UTI.

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Table 1: Antibiotic susceptibility test.

Bacteria	Antibiotic				
	Amekacin (Am)	Ciprofloxacin (Crp)	Erythromycin (E)	Vancomycin (VA)	Gentamycin (Gn)
<i>E. coli</i>	S	S	R	S	S
<i>K.pneumoniaee</i>	S	S	R	R	S
<i>P. aeruginosa</i>	S	S	R	R	R

R= resistance

S= sensitive

Table (2): Chemical qualitative detection of effective grape seed extract groups.

Grape seed extract type	Effectiveness groups									
	Terpene	resins	Essential oils	Steroids	Alkaloids	Glycosides	Phenols	Saponins	Tannins	Coumarine
aqueous extract	-	-	+	-	+	+	+	+	+	-
Alcoholic extract	-	+	+	-	+	+	+	+	+	+

- None present

+ present

Table (3): The rate of inhibition zoon of some bacterial isolates treated with several concentrations of aquatic and alcoholic grape seeds extract.

Bacterial species	Extract	Concentration of grape seeds extract (mg/ml)			
		25	50	100	200
		inhibition zoon (mm)			
<i>Escherichia coli</i>	aquatic	0	2	8	20
	alcoholic	0	10	17	23
<i>Klebsiella pneumonia</i>	aquatic	0	0	12	22
	alcoholic	0	12	19	28
<i>Pseudomonas aeruginosa</i>	aquatic	0	0	6	10
	alcoholic	0	6	10	20

Mg/ml: milligram per milliliter

Mm: millimeter