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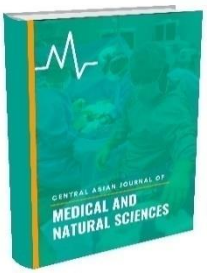

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Antibacterial Activities of Cranberry Extract Against Isolated Bacteria from UTI Patients

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Abstract:

Methods: Cranberry was extracted by means of aqueous and alcohol extraction, in addition to the collection of bacteria samples from patients with urinary tract infection in Imam Hussein Medical city and Alhindyia Hospitals and re-diagnosis by traditional methods (biochemical tests). then testing the sensitivity of antibiotics and measuring the inhibitory effect of the cranberry extract of the bacterial species obtained during this study.

Results: The results of the current study showed that the re-culturing and identifying of bacterial samples obtained from patients with urinary tract infection have shown five bacterial strains (Echerichea Coli, Pseudomonas, Enterobacter, Protus, Staphylococcus aureus). The study showed that the aqueous extract has the highest inhibitory effect against the bacteria and the diameter of the inhibition of 29 mm followed by the extract of methanol and the diameter of inhibition of 28 mm and then the plant Cranberry crude and diameter of inhibition was 19 mm. The sizes of inhibition zones were different according to concentration, We observe an increase in the diameter of the inhibition zone with increased concentration.

Conclusion: In conclusion the, extract of cranberry has antibacterial activity against uropathogens including E. coli, S aureus, Enterobacter, Pseudomonas and Proteus. Therefore.the potential of cranberry product to act as a non-antibiotic alternative for preventing UTI, thereby reducing the total amount of antibiotics prescribed for treatment of UTIs and preventing drug resistance.

Key words: Urinary Tract Infection; Cranberry; Antibacterial activity.

INTRODUCTION

A urinary tract infection (UTI) is a common type of infection that occurs in the urinary tract. The symptoms of a UTI include: pain or a burning sensation during urination (dysuria), a frequent need to urinate, lower abdominal pain, urgency, hematuria, cloudy urine, nausea, vomiting and delirium. (1)

Types of UTI

A UTI develops when part of the urinary tract becomes infected, usually by bacteria. Bacteria can enter the urinary system through the urethra or, more rarely, through the bloodstream.

How common are UTIs?

UTIs are a very common type of infection in women. It is estimated that one woman in three will have a UTI before the age of 24, and that half of all women will have at least one UTI during their lifetime.

UTIs are a lot less common in men. It is estimated that every year in otherwise healthy men, only one in every 2, 000 will develop a UTI, There are estimated 150 million urinary tract infections per year worldwide (2). Urinary tract infection is a bacterial infection that affects any part of urinary tract. In most cases bacteria travel to the urethra and multiply causing kidney infection if not treated (3, 4). The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. Members of this family mostly include *E. coli*, *Klebsiella*, *Enterobacter* and *Proteus*. *Escherichia coli* is one of the most common bacteria capable of causing infection in humans and animals, particularly urinary tract infections(5).

The outlook for most cases of UTI is excellent. The infections are usually mild and will usually resolve within four to five days. Antibiotics can be used to help speed up the recovery time. However, some women find that they experience repeated UTIs, and that they require long-term treatment with antibiotics to prevent the infection returning. In women with recurrent urinary tract infections (UTIs), long-term antimicrobial prophylaxis is indicated (6). This method is effective but can cause adverse reactions and can increase emergence of antimicrobial resistance (7, 8). Microbes have the ability to develop resistance to the drugs becoming drug-resistant organisms (7). An antimicrobial is a kind of drug that destroys or rests the growth of microbes, as bacteria, viruses, fungi, and parasites. Antibiotic resistance :is the ability of bacteria to resistance the effects of an antibiotic, so the bacteria are not destroyed and their growth still occur. Resistant bacteria to the antibiotic lead to rapid growth of microorganisms and spread them in to other organs. Furthermost infection-causing bacteria can become resistant to at least some antibiotics. Therefore, the need for alternative therapies for UTI prophylaxis is evident. Cranberries are one nonantibiotic alternative.

Cranberry fruit.

The scientific name for cranberry plant is *Vaccinium macrocarpon*. The name cranberry is derived from craneberry, first named by early European settlers in America because of their resemblance to head neck and bill of crane. It is widespread throughout the cool temperate northern hemisphere, including northern Europe, northern Asia and northern North America. It is a dwarf shrub and there are four species. Cranberry is used as fresh fruit, juice, sauce and also as medicine (1). Therapeutic value of Cranberries used in trade medicine derives from the presence of mainly vitamin C, dietary fiber, glucose and fructose, flavonoids (flavonols, anthocyanins, and proanthocyanidins), and gallic, benzoic, citric, and oxalic acids. The medicinal effectiveness and safety of cranberry juice/ pills have been critically evaluated (9). Cranberries seem to be the most effective in preventing the adhesion of *E. coli* to uroepithelial cells, which is responsible for 85% of UTI. It has antimicrobial, anti inflammatory and anti tumors activities.(1)

MATERIALS AND METHODS

From 10 to 30 October we are collected Clinical isolates of athogenic (*Echerichea Coli*, Pseudomonas, Enterobacter, Protus, Staphylococcus aureus) isolated from patients with urinary tract infection in Imam Hussein Medical city and Alhindyia Hospitals.Then, the isolates were re- identified in our Lab.

by biochemical tests includes (gram stain, culturing on blood agar, macConkey agar, manitol agar, EMB agar and the catalase, oxidase, KOH, IMViC tests). (10) Isolates maintained on brain heart infusion agar and stored at 4 Co, and were sub cultured once every two-week (11).

Extraction of Cranberry

Organic Extraction

Methanol extraction of the cranberry was carried out by using methanol according to method described by (12). This was obtained by using 10 g of dried fruit placed in 100 ml of methanol in a conical flask, and then kept on a rotary shaker at 190 - 220 rpm for 24 h. After 24 h, then filtered and centrifuged at 4500 rpm for 15 min. The supernatant was collected and the solvent was evaporated by using incubator 40 °C to get rid of methanol and then stored at 4 °C in airtight bottles for further studies.

Aqueous Extraction

For aqueous extraction, 10 g of dried powder was placed in 100 ml de-ionized water in beakers and heated for 10 min on a magnetic stirrer hotplate until the temperature reached 95 °C. Subsequently, the mixtures were allowed to cool for 10 min to increase extraction of active compounds. Extracts were then filtered through filter paper (What man size 41) to remove smaller particles using a Buchner funnel. Each final extract was stored in a dark screw-cap sterile container (this is done because phenolics are photo- sensitive) and then stored at 4°C for 24 h before use (13).

Antibiotic sensitivity test

Disk diffusion test was used to determine the sensitivity of isolated bacteria to antibiotics according to Morello et al. (2006) (14), and the antibiotic disk that are used as follow (penicillin G 10 units; Gentamicin10µg; Tetracycline 30µg; Erythromycin15µg, Amikacin 30µg; Levofloxacin 5µg; Cefoxitine 30µg ;Norfloxacin 10; Clindamycin 2 µg; Vancomycin30µg, Ceftriaxone 5µg, meropenem, Nalidixic Acid, Tigecycline and Ampiciline Sulbactum).

Antibacterial Test

for assessing the antibacterial activity of the prepared extract. The bacterial suspension was prepared by transferring 2-3 colonies with the same phenotypic characteristics growing on the Nutrient Agar

medium to tubes containing the crystalline saline solution.Then compared the tubes with a 0.5 McFarland standard, which gives a approximate number of cells to 1.5×10^8 colony / ml.

Determination of inhibitory efficacy of cranberry extract.

The inhibitory efficacy of the cranberry extracts against the bacterial isolates obtained in this study were tested with concentrations of 1, 0.5 and 0.25, 0.125 and 0.0625) g / mL according to the agar gel diffusion method (Egorove, 1985) as follows:(15)

1. Add 0.1 ml of the bacterial suspension to the Muller Hinton agar plates. Spread it on the surface of the plates by cotton swab and leave the plates for drying.
2. After drying the plate at 37 ºC for 30 minutes, make a well on the surface of the plates by using a sterile crock borer in appropriate diameter(6) mm under aseptic condition.
3. Place 60 micro liters of each concentration of extract in each well, and then incubated in the incubator at 37 ° C for 24 hours.
4. The diameter of inhibition zone of bacteria (mm) was measured using the ruler after the completion of incubation period.

Results :

The results of the re-culturing and identifying of bacterial samples obtained from patients with urinary tract infection have shown five bacterial strains (Echerichea Coli, Pseudomonas, Enterobacter, Protus, Staphylococcus aureus) as shown in the table (1, 2) below :-

Table 1: Shows the morphological and biochemical tests of *S.aureus*bacteria

	Tests	Results
1_	Gram stain	+
2_	The appearance	Spherical cells in clusters
3_	Growth condition	Facultative anaerobic
4_	Catalysis test	+
5_	Oxidase test	-
6_	DNase test	+
7_	Mannitol fermentation	+
8_	Coagulation test	+
9_	Hemolysis on blood	Beta hemolysis

+ positive result, - negative result

Table 2: Shows the morphological and biochemical tests of *G-ve* bacteria

	Results / **Tests**	***Pseudomonas***	***E.coli***	***Enterobacter***	***Proteus***
1-	Gram stain	_	_	_	_
2-	The appearance	Rod cells	Rod cells	Rod cells	Rod cells
3-	Growth conditions	Obligate anaerobic	facultative anaerobic	facultative anaerobic	facultative anaerobic
4-	Catalysis test	+	+	+	+
5-	Oxidase test	+	_	_	_
6-	Motility tsest	+	+	+	+
7-	Lactose fermentation	_	+	+	_

8-	Selective media EMB	Not lactose fermenter	+ (green metallic)	pink coloration with slight darkening	Gray colonies
9-	Indol test	_	+	_	--/+
10-	MR test	_	+	_	+
11-	VP test	--/+	_	+	_
12-	Citrate test	+	_	+	--/+
13-	Urea test	_	_	+	+

+positive result, - negative result

A larger amount of cranberry extract was obtained when extracted with water from methyl alcohol extraction as shown in Table (3):-

Extraction solvent	Original weight of powder (g)	weight of extract (g)
Methanol	5	2
water	5	3

The isolated bacterial showed wide differences in their susceptibility to the tested antibiotics. We note from the results listed in table (4) that *E. coli* had the highest antibiotic resistance ratio at 63.6 %, while the sensitivity ratio was 27.2% and the intermediate ratio was 9 %, Followed by *Pseudomonas* bacteria where the resistance ratio is 55.5% while the sensitivity ratio was 44.4 %, Followed by *Enterobacter* bacteria where the resistance ratio is 50% while the sensitivity ratio was 20 % and the intermediate ratio was 30 %, Followed by *Proteus* bacteria where the resistance ratio is 40% while the sensitivity ratio was 60 %, and finally *Staph.aureus* bacteria where the resistance ratio is 33.3 % while the sensitivity ratio was 55.5 % and the intermediate ratio was 11.1 %.

Table 4 : Antibiotic sensitivity and resistance of the isolated bacteria.

Type of bacteria / Antibiotics	Diameter of inhibition zone (mm)				
	E.coli	***Pseudomonas***	***Proteus***	***Enterobacter***	***Staph.aureus***
Pencillin G	**6 resistens**	**6 resistens**	**6 resistens**	**6 resistens**	
Cefoxitin	**20 intermediate**	**6 resistens**	**20 Sensitive**	**14 resistens**	**16 Intermediate**
Levofloxacine	**8 Resistens**	**35 Sensitive**	**20 Sensitive**	**22 Sensitive**	**20 Sensitive**
Norfloxacine	**6 Resistens**	**38 Sensitive**	**22 Sensitive**	**13 Intermediate**	**19 Sensitive**
Ampiciline Sulbactum	**12 resistens**	**6 resistens**	**10 resistens**	**6 Resistance**	**6 Resistance**
Ceftriaxone	**7 Resistens**	**15 resistens**	**37 Sensitive**	**6 Resistance**	**25 Sensitive**
Amikacine	**18 Sensitive**	**20 Sensitive**	**18 Sensitive**	**16 Intermediate**	**20 Sensitive**
Nalidxic Acid	**6**	**6**	**6**	**18**	

	Sensitive	resistens	Resistens	Intermediate	
Meropenem	**26 Sensitive**	**23 Sensitive**	**24 Sensitive**	**24 Sensitive**	**25 Sensitive**
Clindamycine	**7 resistens**		**6 resistens**	**6 Resistance**	**6 Resistance**
Tetracycline	**6 resistens**				**6 resistens**

Antibacterial Activity

Different concentrations of Cranberry extract and the Cranberry crud that were used in agar diffusion assay caused different degrees of zones of inhibition against isolated bacteria (*Echerichea Coli*, *Pseudomonas*, *Enterobacter*, *Protus*, *Staphylococcus aureus)* Figure (2). The sizes of inhibition zones were different according to concentration, We observe an increase in the diameter of the inhibition zone with increased concentrationF. The highest inhibition diameter at concentration 1 g / ml with decreasing inhibition zones gradually at concentrations(0.5, 0.25, 0.25, 0.265) g / ml.

Table (5): Show the inhibitory activity of the crud cranberry plant and the extracted against bacteria isolated from urinary tract infection

Bacteria	*Eschrichea* Coli	*PROTEUS*	*Staphylococcus aureus*	*Enterobactar*	*Pseudomonas*
Concentration gm	Inhibition zone Diameter (mm)				
Crude	15	16	17	11	19
1	14	14	14	10	14
0.5	10	12	12	0	12
0.25	0	11	11	0	0
0.125					
Methanol Extraction	28	17	25	0	17
1	23	15	22	0	13
0.5	19	12	15	0	11
0.25	14	10	12	0	0
0.125	10	0	11	0	0
0.062					
Aqueous Extract					
1	29	18	22	18	22
0.5	26	16	18	15	19
0.25	25	13	14	12	15
0.125	17	13	12	0	11
0.062	11	0	8	0	0

The results showed a decrease in the inhibitory activity of the cranberry crud with the lowest inhibition zones for the tested bacteria than the extract.

The Cranberry extract with water as a solvent showed the highest inhibitory rate than methanol use, as shown in Table (5).

There is also a difference in the degrees of zones of inhibition for different bacterial species, The results showed that *E. coli* was more sensitive to Aqueous extract of cranberry than methanol and crud in all the concentrations using in this study, in diameter of zone of inhibition 29 mm, followed by bacteria *Pseudomonas* and *Staph. aureus* with diameter of zone of inhibition 22 mm, followed by bacteria *Proteus and Enterobacter* with diameter of zone of inhibition 18 mm. The results also showed that the alcohol extract had no inhibitory effect against bacteria *Enterobacter*, while *E. coli* showed the highest inhibition zone of diameter 28 followed by *Staph.aureus*, then *Pseudomonas* and Proteus bacteria with inhibition diameters (25 and 17 mm) respectively.

While we note the least inhibitory effect of bacteria when using the cranberry crud, where showed the bacteria *Pseudomonas* highest diameter of zone of inhibition 19 mm, followed by bacteria *Staph.aureus*, *Proteus*, *E. coli*, *Enterobacter* with inhibition diameters (17, 16, 15, 11) mm respectively.

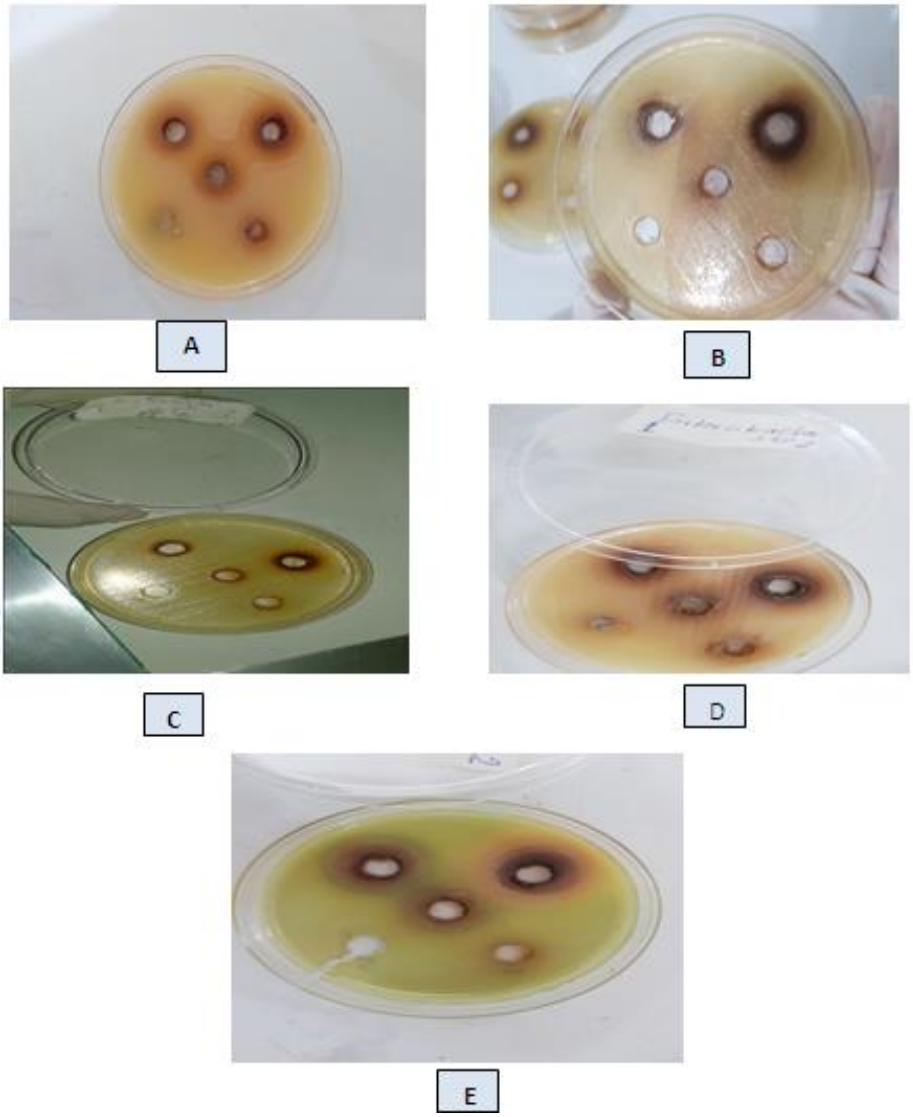

Figure 2: Aqueous extract on **A**-E.coli, **B-** Proteus, **C**- *Staph.aureus*, **D**- *Enterobacter*, **E**- *Pseudomonas.*The concentration on the wells upper left is 1 g/ml and the upper right is 0.5 g/ml, in the middle is 0.25 g/ml, and the bottom right is 0.125 g/ml and the bottom left is 0.265 g/ml

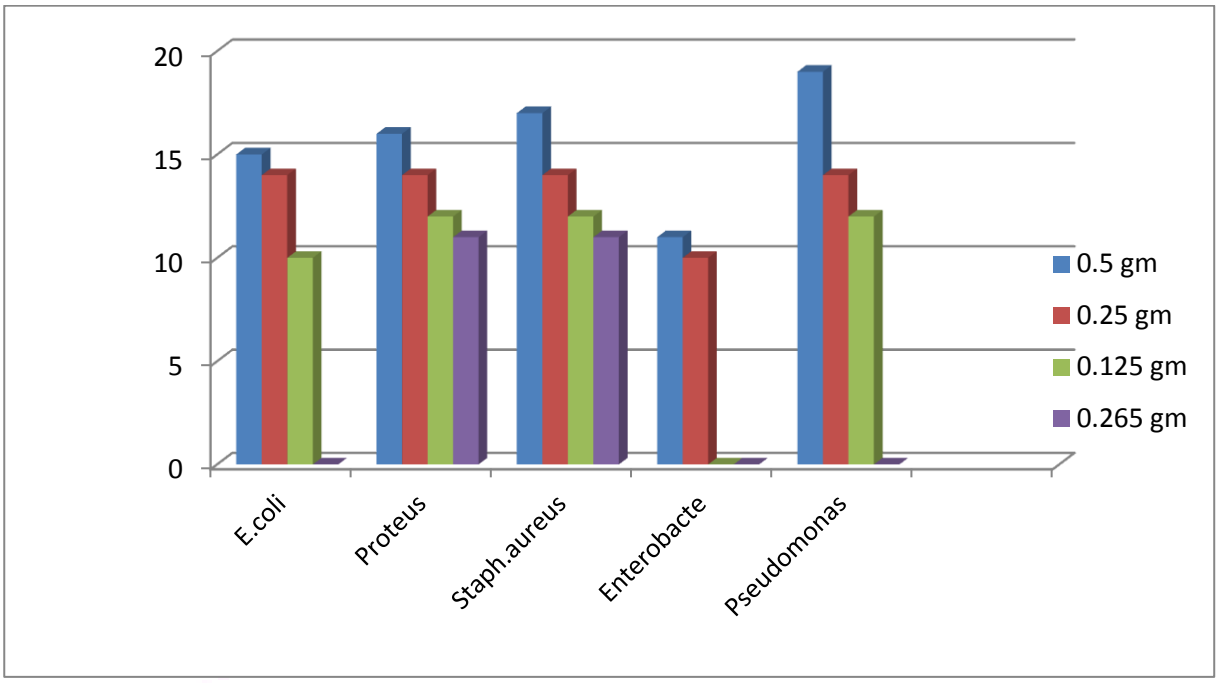

Figure 3: Inhibitory effect patterns of the cranberry crud on isolated bacteria.

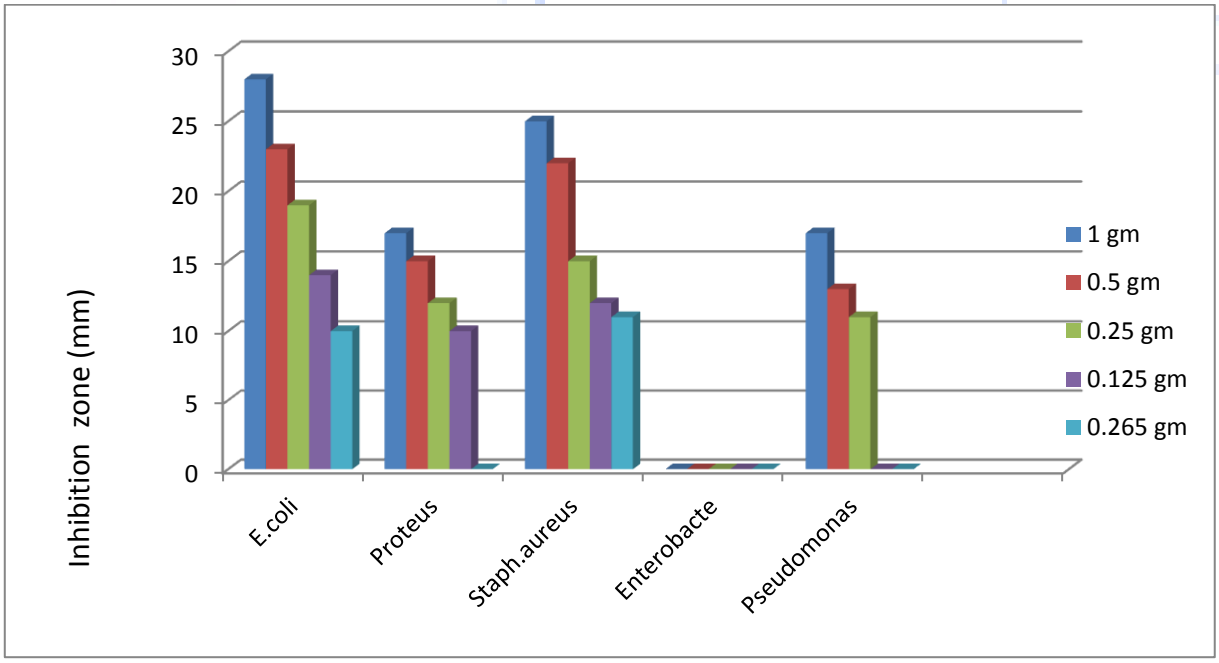

Figure 4: Inhibitory effect patterns of the cranberry methanol extract on isolated bacteria.

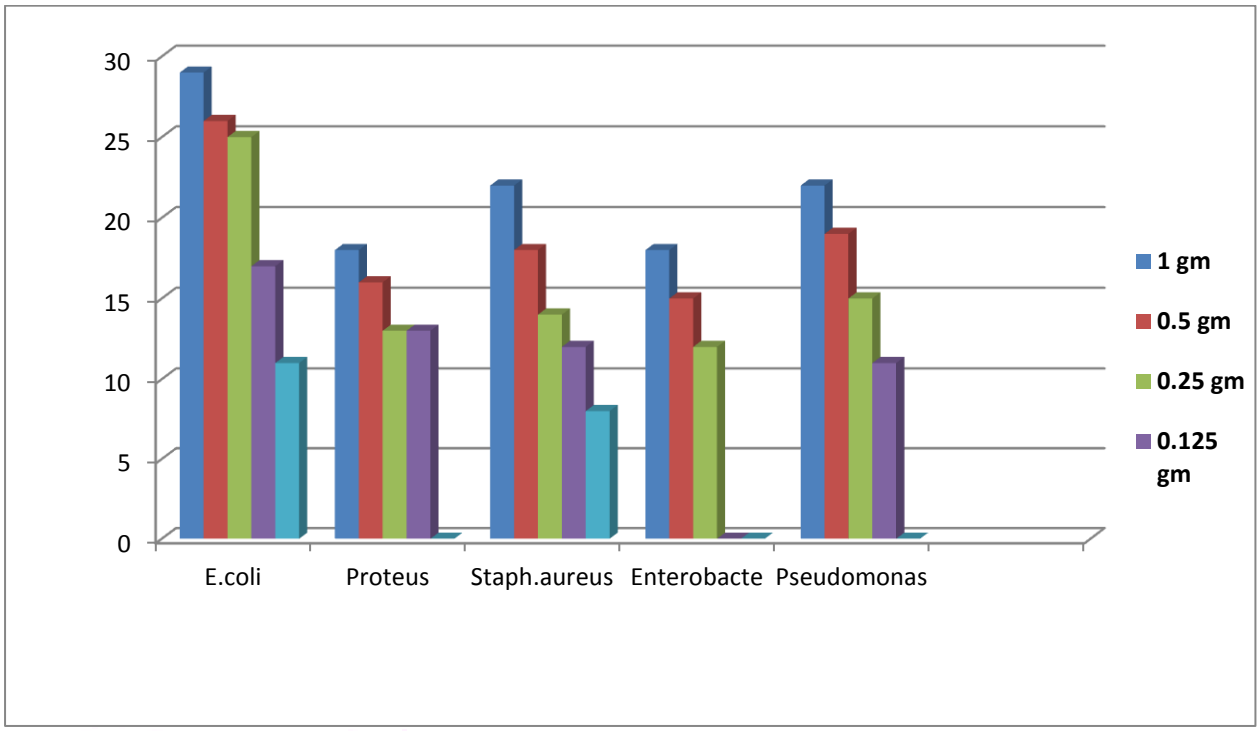

Figure 5: Inhibitory effect patterns of the cranberry Aqueous extract on isolated bacteria.

Discussion

There are many studies regarding antibacterial activity and usage of cranberry extract in treatment of UTI. In the present study, the antibacterial activity of cranberry extract on *E. coli*, *Enterobacter*, *Pseudomonas*, *Proteus* and *S. aureus* was studied Figure 2.The results obtained from this study showed that the methanol and aqueous extract of this plant inhibits the growth of the test isolates at varying concentration. Antibacterial activity of various concentrations of cranberry extract against different strains of urine isolates are shown in Table 5.

These study suggests that concentrated cranberry juice has antibacterial activity especially on uropatogens, Other study by Magaarinos et al showed that cranberry juice has inhibitory effects against pathogenic microorganisms including *E. coli*, *Salmonella* spp, *Listeria monocytogenes.P. aeruginosa* and *S. aureus*. (16) Other study also has shown that cranberry extract reduce biofilms formation on uroepthelial cells (17 ; 18).

Clinical trials have shown the effectiveness of cranberry in treatment of urinary tract infections. The explanation for the usefulness of cranberry juice was thought to be the excretion of hippauric acid in the urine. Hippauric acid is a strong bactriostatic agent. Hippuric acid has potential power to acidify urine (19; 20; 21).

More recently attention has turned to the adherence of bacteria to the epithelial cells of the urinary tract, which is necessary if the bacteria cause infection. Sobota (1984) was the first to suggest that use of cranberry juice inhibit bacterial adherence to urinary tract epithelial cells.(22)

A recent study showed that regular consumption of cranberry juice was also effective in cases in patients with UTI caused by antibiotic resistant bacteria (23).

Recommendation :

1. Repeat the study in the future with larger sample size to achieve more accurate and dependable results.

2. Test bacterial isolates of the same type as *E. coli*.
3. Test another solvents in the cranberry extraction such as acetone, Ethanol and in different concentration.

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