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The antimicrobial effects of green tea and lemon juice on *Escherichia coli* isolated from patients with urinary tract infection in holy Karbala city

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ABSTRACT: Urinary tract infections (UTIs) are the second most common type of infection found in any organ system, and the most common type of nosocomial infection. The strains of *Escherichia coli* that infect the urinary tract are categorized as uropathogenic *E. coli*. The antimicrobial agents that have traditionally been used to treat UTIs (β-lactams, fluoroquinolones, trimethoprim–sulfamethoxazole, nitrofurantoin, etc.) are becoming less effective, According to the reports of many researchers, antibacterial resistance is a worldwide growing problem. Cephalosporin's are useful for UTI's which do not respond to other drugs, can cause side effects that may be serious or even cause death.

Green tea is derived from non-fermented leaves of the *Camellia Sinensis* plant, Green tea has been a favored drink, traditionally, in Asian countries. Because of studies that have shown the potential health benefits of green tea. A study found that citrus juices enable more of green tea's unique antioxidants to remain after simulated digestion, making the pairing even healthier than previously thought.

The bacteria strains use in this study are part of a research collection of *E. coli* isolate from UTI cultures during the year of 2016. A total of 10 *E. coli* isolates are collect from urine specimens submit of selected Al- Hussein medical city patients during one month period throughout November 2015. The isolates are further processes by standard methods to identify as E. coli isolates. This method included the morphological properties and biochemical tests.

The aqueous and alcoholic extracts were prepared by Ahmed's method then the filtered extracts poured in clean and sterile petri dishes and let to dry in the oven for two 2-3 days at a temperature 45-50 C˚, The concentrations (25,50,75,90,100) % were prepared from the stuck for each aqueous and alcoholic for the green tea and green tea with lemon extracts.

The results showed that the best antimicrobial activity found in concentration of 90% aqueous green tea and at 90% of alcoholic green tea with lemon.

KEYWORDS: green tea, Lemon juice, Escherichia Coli, Urinary tract and Antimicrobial.

1 INTRODUCTION

Urinary tract infections (UTIs) are the second most common type of infection found in any organ system, and the most common type of nosocomial infection[1]. These UTIs are responsible for over eight million doctors' visits per year in the U.S[2], and result in medical costs of over six billion dollars worldwide per year[3][4]. Most UTIs (80–90%) are the result of infections with *E.coli*[4]. Non-pathogenic strains of *E. coli* are an important part of the normal flora in the human intestinal tract.

The strains of *E. coli* that infect the urinary tract are categorized as uropathogenic *E. coli* [5] (UPEC). The UPEC are able to produce special surface proteins (adhesins) that allow them to attach to and invade the epithelial cells that line the urinary bladder[3]. If the infection is not eradicated while it is in the bladder (uncomplicated UTI), some strains of UPEC may then travel up the ureters to the kidneys and cause even more severe infections (complicated UTIs), which can lead to renal damage and possibly renal failure[5].

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The antimicrobial agents that have traditionally been used to treat UTIs (β-lactams, fluoroquinolones, trimethoprim–sulfamethoxazole, nitrofurantoin, etc.) are becoming less effective [7] [6], According to the reports of many researchers, antibacterial resistance is a worldwide growing problem [8].Cephalosporin's are useful for UTI's which do not respond to other drugs, CIPRO can cause side effects that may be serious or even cause death. Characteristics of individual cephalosporins may vary. Cephalexin, cephradine, cefaclor and cefadroxil may be used with caution during pregnancy and lactation[9].

These challenges have been receiving growing interest to find alternative antimicrobial agents from plant extracts that need to be developed and used to control multidrug-resistant bacteria[10][11][12].

Green tea is derived from non-fermented leaves of the *Camellia sinensis* plant. Oolong and black tea are made from fermented leaves of the same plant. Green tea has been a favored drink, traditionally, in Asian countries. Because of studies that have shown the potential health benefits of green tea, it is now gaining worldwide popularity as a drink that is important in preventative medicine. Studies using green tea have shown it to have potential benefits, most notably in: cardiovascular disease, cancer, diabetes, obesity, oral health, bone health, and cognitive function[13][14][15]. In addition, green tea has been shown to have antimicrobial effects[10][16][17][18].

Recent studies suggest that green tea may contribute to a reduction in the risk of cardiovascular diseases and some forms of cancer as well as promotion of oral health and other physiological functions such as anti-hypertensive effect, body weight control, ultraviolet protection, bone mineral density increase and neuro-protection power[19][20].

A study found that citrus juices enable more of green tea's unique antioxidants to remain after simulated digestion, making the pairing even healthier than previously thought[21].

1.1 INTERACTION BETWEEN LEMON JUICE AND GREEN TEA

Lemon juice is believed to have antimicrobial properties in many cultures of the world. In the Caribbean, South America and Africa, lemon juice is believed to be effective against diphtheria and upper respiratory tract infections [22]. In South Africa, lemon juice has been used in the treatment of oral thrush in HIV/AID patients [23] In many parts of the world, lemon juice is also used as sanitizers to remove food borne pathogens from fresh fruits, vegetables and fish [24][25]. Studies have shown that concentrated or freshly squeezed lemon juice has antibacterial activity against *Vibrio* species[25], *Salmonella typhimurium* [24], *Pseudomonas aeruginosa* and *E. coli* [26].

A study found that citrus juices enable more of green tea's unique antioxidants to remain after simulated digestion, making the pairing even healthier than previously thought[21].

2 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 THE USED DEVICES

Table 1: The devices that used in this study

Company	The device name	NO.
Sartorious – Germany	Sensitive Balance	1
Fisher Scientific – Germany	Incubator	2
Motic – Germany	Microscope	3
Yx – 280 B – China	Auto Clave	4
Jeiotech – Korea	Hood	5
LG – India	Refrigerator	6

2.1.2 MEDIA

Sterilized culture media into autoclave at temperature 121 c˚ under pressure 15 pound/inch ² for 15 min which are Macconkey agar, Mueller Hinton agar, Nutrient broth, Eosin methylene blue agar and Blood agar. Indicators are Gram's stain kit.

2.2 METHODS

2.2.1 BACTERIA STRAIN

The bacteria strains use in this study are part of a research collection of *E. coli* isolate from UTI cultures during the year of 2016. *E. coli* isolates are collect from urine specimens submit of selected Al- Hussein medical city patients during one month period throughout November 2015. The isolates are further processes by standard methods to identify as *E. coli* isolates, This methods are Gram stain kit, indole test and methyl- red reagent (IMVic test)

2.2.2 ANTIBIOTIC SENSITIVITY TEST

According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar using Kirby Bauer disk diffusion method[27]. The antimicrobial agent tested were ciprofloxacin (5 µg).

2.2.3 PREPARATION OF PLANT EXTRACTS

A commercial green trade mark variety Al-Wazah, bought from a supermarket in Karbala, is use in the study. Prepared the extracts according to Ahmed method [28]

2.2.3.1 AQUEOUS EXTRACTS

Aqueous extracts prepared by mixing 10gm from the Camellia sinensis non fermented leaves for the green tea extract and 5gm of the Camellia sinensis non fermented leaves with 5ml of natural lemon juice for the green tea and lemon extract with 200 ml distilled water in volumetric flask capacity of 1000 ml and left stuck with stirring in a rocking water bath for 24 hours at a temperature of 40 C˚ later the extracts filtered through several layers of sterilize gauze first then through filter paper.

2.2.3.2 ALCOHOLIC EXTRACT

For the alcoholic extract mixing 10gm from the Camellia sinensis non fermented leaves for the green tea extract and 5gm of the Camellia sinensis non fermented leaves with 5ml of natural lemon juice for the green tea and lemon extract with 200 ml of 70% ethanol in volumetric flask capacity of 1000 ml and left stuck with stirring in a rocking water bath for 24 hours at a temperature of 40 C˚ later the extracts filtered through several layers of sterilize gauze first then through filter paper.

Then the filtered extracts poured in clean and sterile petri dishes and let to dry in the oven for two 2-3 days at a temperature 45-50 C˚ as shown in fig. (1)

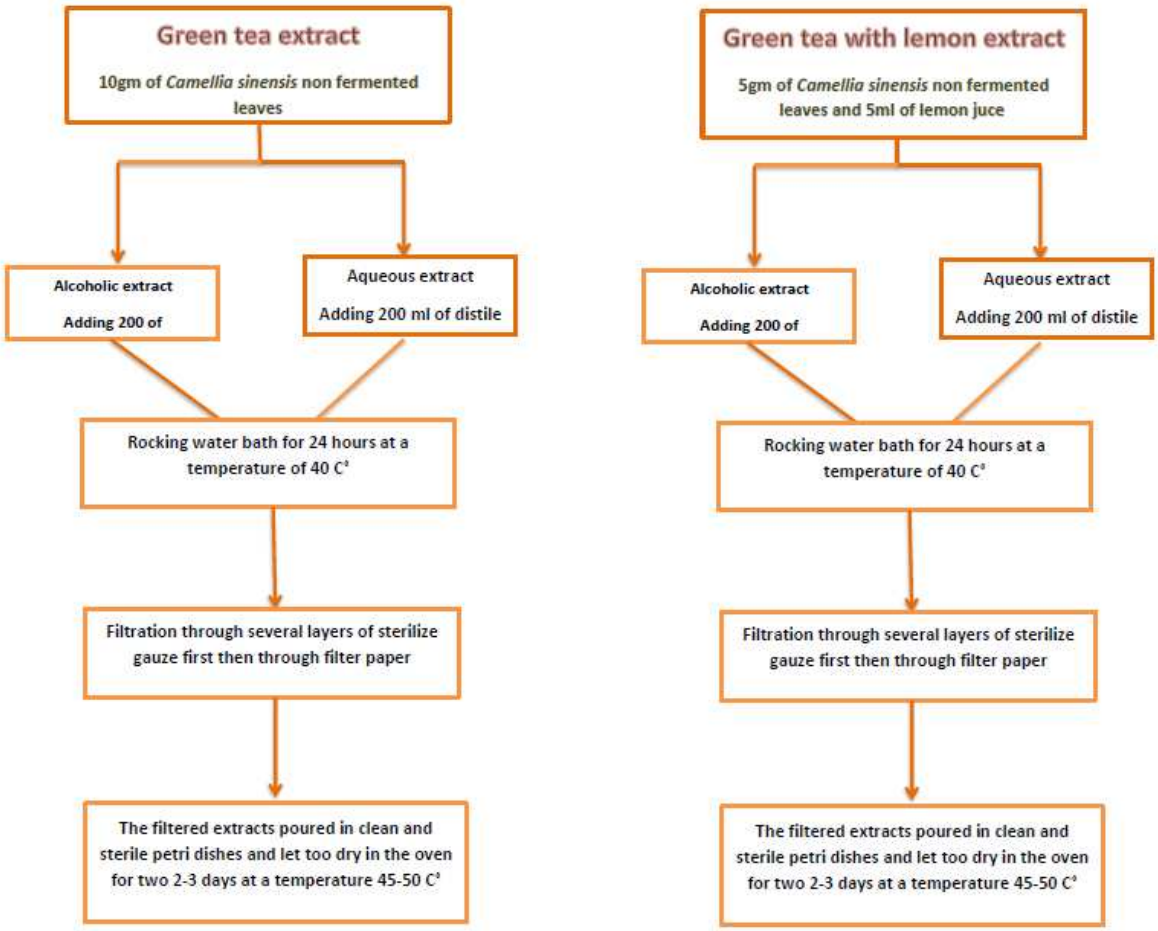

Fig. 1: The methods of Preparation of plant extracts

2.2.3.3 PREPARING EXTRACTS DILUTION SERIES

The concentrations (25,50,75,90) % were prepared from the stuck for each aqueous and alcoholic for the green tea and green tea with lemon extracts.

2.2.4 DETERMINATION OF THE BEST BACTERIAL GROWING INHIBITORY CONCENTRATION

The best bacterial growing inhibitory concentration of the green tea extract was determined by the following:-

1- The bacterial cultures were refreshed on nutrient broth and incubated for 24 hours at temperature 37cº.
2- Microbial Suspensions were serially diluted to 10^{-4} (McFarland standards number four). 0.1 ml of the suspensions were spread on Mueller Hinton agar by the L shaped.
3- Then pores with 5 mm diameter are made by the Piercing Cork in the rate of 5 pores for each plate.
4- 0.4 ml of each concentration of the extracts were lay down in pores in the rate of two plates for each concentration.
5- The plates were incubated for 24 hours at temperature 37cº,then the inhibition zones were measured .
6- To ensure the results a second method were done by pour plates with 0.1 ml of bacterial suspensions in sterilize plates then Mueller Hinton agar poured and mixed in ∞ movement on the bench.
7- One plate were incubated for 24 hours at temperature 37cº to be the control .
8- Then a pores made with the piercing cork made in the plates and the filled with 0.4ml of the extracts.
9- Then the growth of bacteria were measured compering to the control plate as the antimicrobial effect of the extracts as heavy, intermediate and weak.

2.2.5 SYNERGISTIC ACTION

To measure the interaction between the extracts and the used antibiotic pour plate method done by pour plates with 0.1 ml of bacterial suspensions in sterilize plates then Mueller Hinton agar poured and mixed in ∞ movement on the bench, after drying 0.1 ml of the effective concentration of the extracts were spread on the plates and let to dry then the antibiotic discs were distributed on the plates , then the plates were incubated for 48 hours at temperature 37 cº.

After the incubation the growth of bacteria measured and compared to control plate without the extracts. (Control plate made of pour 0.1ml of suspension and Mueller Hinton agar in sterilize plate and le to dry then antibiotic discs were distributed.

Fig. (2): Part of the practical side of the preparation of the extracts

3 RESULTS

3.1 ISOLATION AND IDENTIFICATION OF *E.COLI*

Isolates of *E.coli* were obtained on blood agar from patients with urinary tract infection.

3.1.1 CULTURE CHARACTERISTICS

Streak plate isolation of E. coli on MacConkey Agar grown for 24 hours at 37 c. *E. coli* demonstrates strong lactose fermentation indicated by the bright pink halo bile precipitant around the colonies and pink colony growth. As shown in fig. (3).

Fig. (3): E.coli on MacConkey agar

Streak plate isolation of *E. coli* on Eosin-methylene blue (EMB) agar plate showing good growth of dark blue-black colonies with metallic green sheen indicating vigorous fermentation of lactose and acid production which precipitates the green metallic pigment. As shown in fig. (4).

Fig. (4): E.coli on Eosin-methylene blue agar

3.1.2 MICROSCOPIC EXAMINATION

A microscopic examination of gram stained slide showed that *E. coli* is a Gram negative non spore forming rod shaped bacterium. As shown in fig. (5).

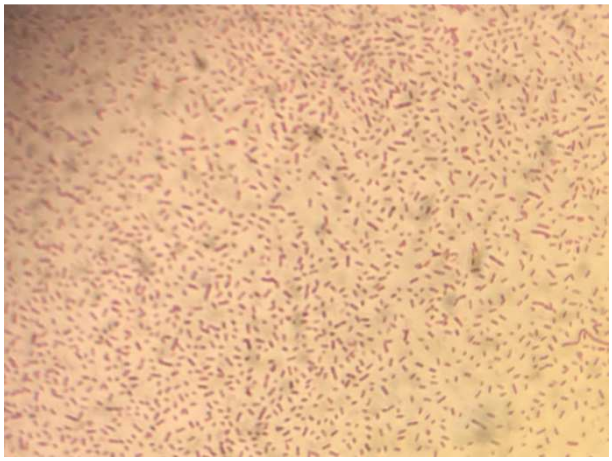

Fig. (5): Microscopic examination of E.coli gram stained

3.1.3 BIOCHEMICAL TESTS

Results of an IMViC series done on *E. coli* after 24-hour incubation at 37°C, showed a positive indole test in tryptone broth. The positive result is indicated by the red layer at the top of the tube after the addition of Kovács reagent.as shown in fig. (6).

Fig. (6): Indole test

And a positive methyl red test as indicated by the red color after the addition of methyl red reagent.

And a negative Voges-Proskauer test as indicated by the lack of color change after the addition of Barritt's A and Barritt's B reagents. Also a negative citrate that result as indicated by the lack of growth and color change in the tube.

Table (2): Results of microscopic and morphological and biochemical tests of E.oli isolated according to Bergey's manual

Results of microscopic and morphological and biochemical tests of *E.oli* isolated	
Characteristics	**Result**
Gram Staining	Negative
Shape (Cocci/Diplococci/Rods)	Rods
Motility (Motile / Non-Motile)	Motile
Capsule (Capsulated/Non-Capsulated)	Non-Capsulated
Spore (Sporing/Non-Sporing)	Non-Sporing
Flagella (Flagellated/Non-Flagellated)	Flagellated
Indole	Positive (+ve)
Methyl red	Positive (+ve)
Voges proskauer	Negative (-ve)
Citrate	Negative (-ve)

3.2 SENSITIVITY TEST

Inhibition zone with diameter of 28mm appeared due to the effective antimicrobial agent ciprofloxacin. As shown in figure (6).

Fig. (7): Sensitivity test

3.3 THE BEST BACTERIAL GROWING INHIBITORY CONCENTRATION OF GREEN TEA AND GREEN TEA WITH LEMON DETERMINATION

The results showed that the best antimicrobial activity found in concentration of 90% aqueous green tea and at 90% of alcoholic green tea with lemon as shown in table no. (3) And table no. (4).

Table (3): The results of effects of green tea with lemon extracts at deferent concentration in bacterial growth

Concentrations of extract	100%	90%	50%
Alcoholic	W	H	I
Aqueous	I	W	I
H for heavy, I for intermediate, W for weak			

Table (4): The results of green tea extracts at deferent concentrations in bacterial growth

Concentrations of extract	**100%**	**90%**	**50%**
Alcoholic	w	W	I
Aqueous	I	I	I
I for intermediate, W for weak			

The number of grown colonies were counted to and the most inhibitory action found in the extracts of green tea with lemon at concentration of 90% as shown in table no. (8) and figure no. (5),(6),(7).

Table (5): The effects of the 90% concentrations of the extracts on the number of grown colonies

Type of extracts	**control**	**Aqueous green tea**	**Alcoholic green tea**	**Aqueous green tea with lemon**	**Alcoholic green tea with lemon**
No. of grown colonies	≤900	107	667	248	6

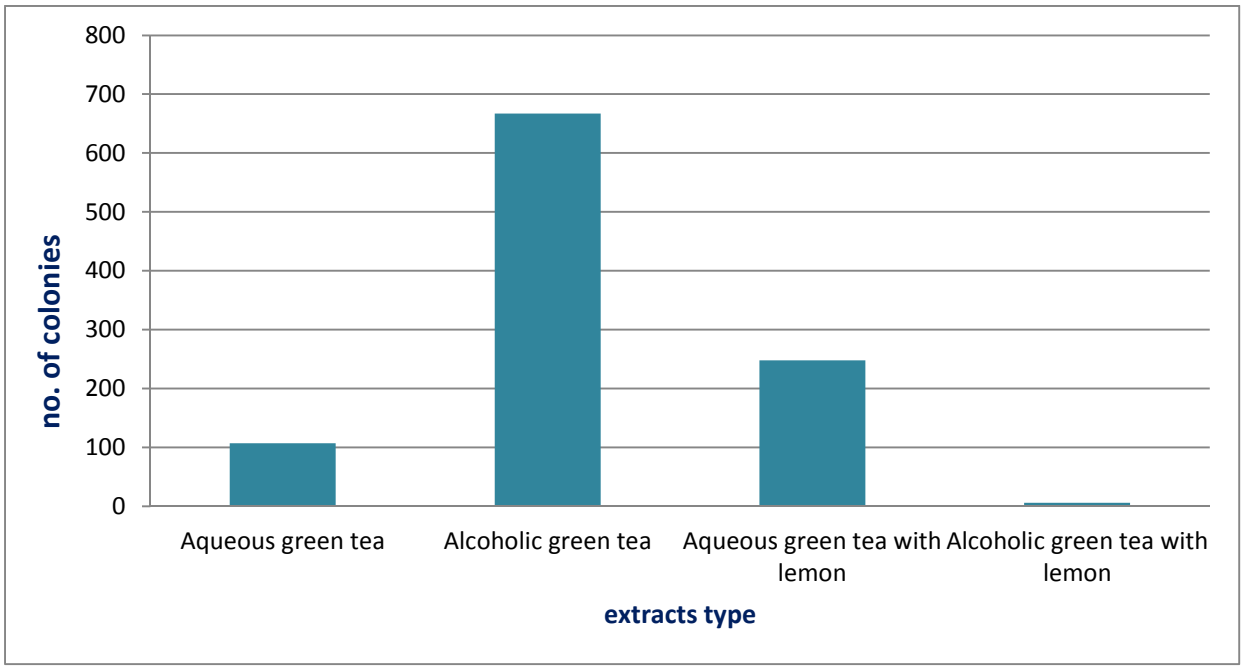

Figure (8) the effects of the extracts on number of grown colonies at concentration 90%

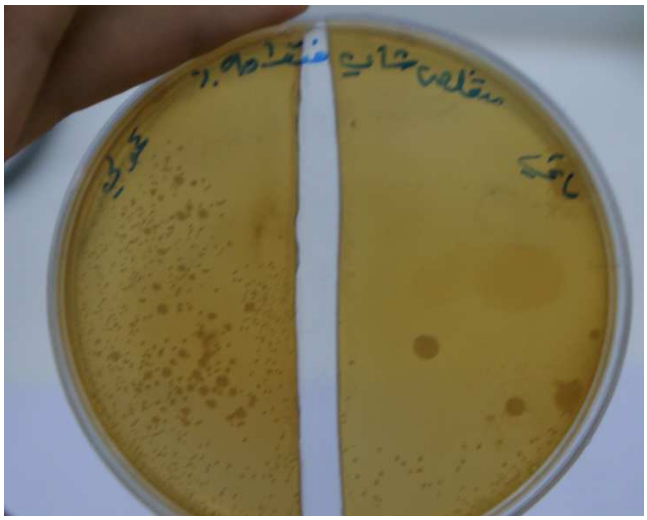

Fig. (9): The effects of the 90%Concentrations of green tea extracts

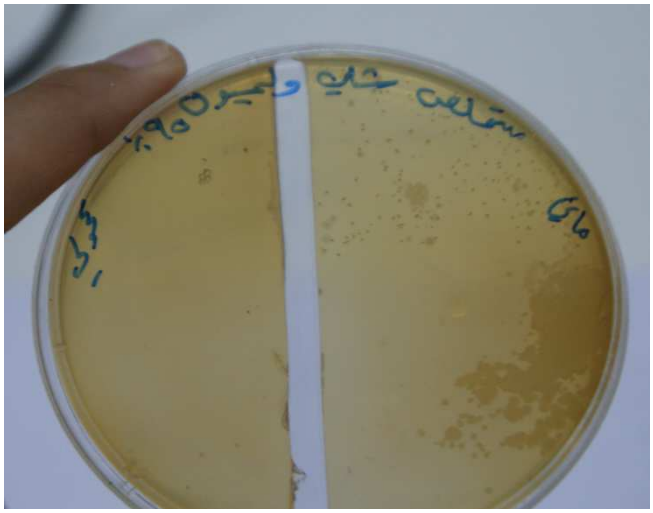

Fig. (10): The effects of the 90%Concentrations of green tea with lemon

Table (6): The Synergistic effects of the extracts at concentration of 90% in the growth of bacteria and comparing the results with the control.

Extract type	control	Antibiotic use	Aqueous green tea	Alcoholic green tea	Aqueous green tea with lemon	Alcoholic green tea with lemon
Growth type	V.H	I	H	W	I	W
V.H for very heavy , H for Heavy ,I for intermediate, W for weak						

Table (7): The Synergistic effects of the extracts at concentration of 90% in the in the number of grown colonies.

Extract type	Antibiotic control	Alcoholic green tea	Aqueous green tea	Alcoholic green tea with lemon	Aqueous green tea with lemon
Number of colonies	**170**	**140**	**130**	**126**	**160**

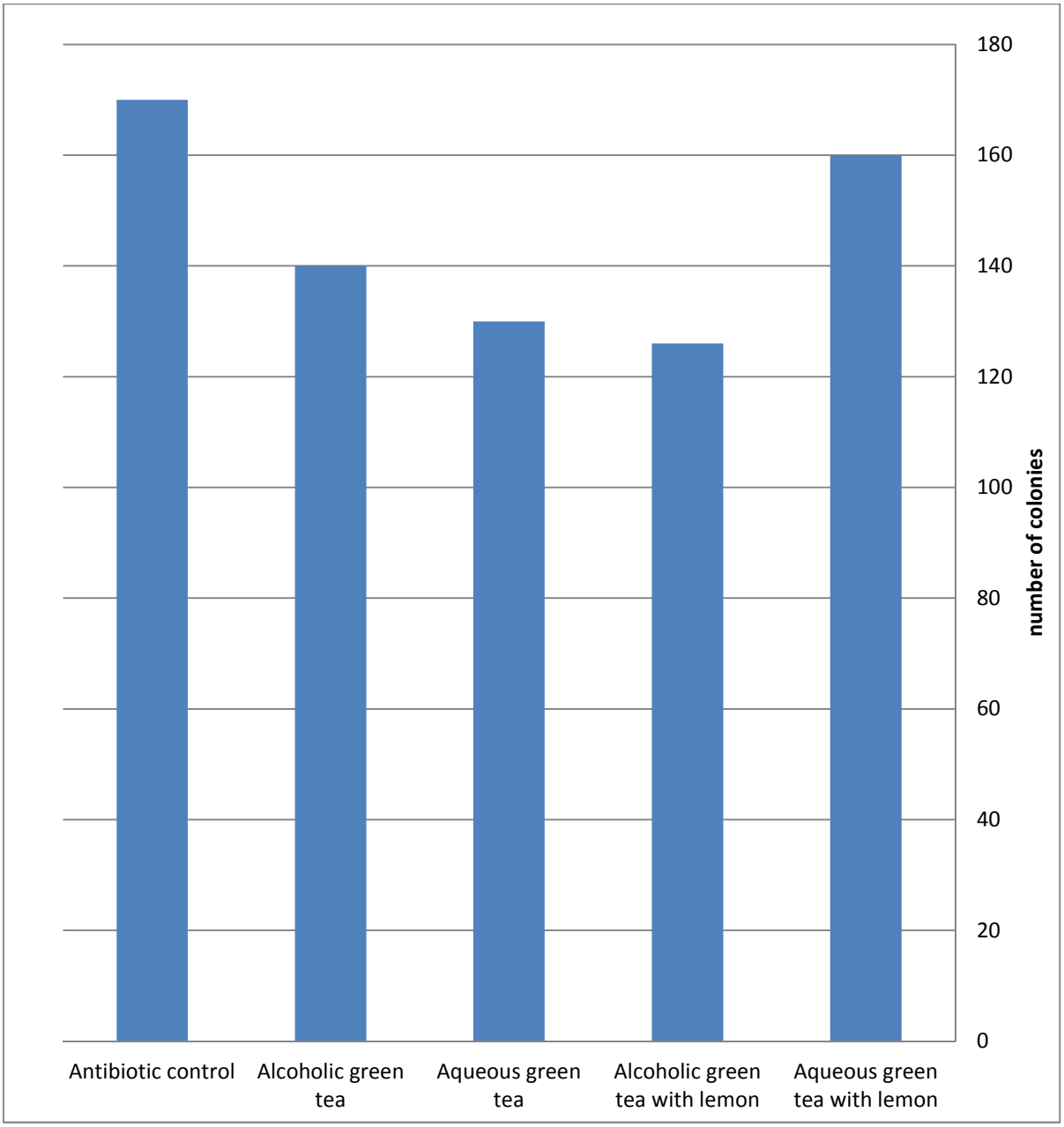

Figure no. (11) the synergistic effect of the extracts at 90% concentration in the number of grown colonies

4 DISCUSSION

The results of confirmatory tests on the isolates that were taken from al Hussein medical city patients with urinary tract infection for all the properties that *E.coli* characteristic with were positive.

After refreshing it on nutrient broth the fourth McFarland standard was taken and cultivate on Mueller Hinton agar by taking 0.1µm of the specimen and adding different concentrations of green tea and green tea with lemon extracts, the results showed that the best inhibitory concentration to inhibit the growth of bacteria were 90% for the alcoholic green tea with lemon extract in the same time the same concentration give the best inhibitory rate in the green tea aqueous extract.

To confirm the results the concentration of 90% was selected and redistributed on bacterial grown on Mueller Hinton agar and after incubation the number of grown colonies were counted for the aqueous and alcoholic extract at concentration of 90% for each of the green tea and green tea with lemon extracts, the green tea with lemon alcoholic extract shows the

best results in elimination growing bacteria since only 6 colonies where grown comparing to the control that exceeded 900 colonies.

After studying and measuring the inhibitory effect of the antibiotic ciprofloxacin on *E.coli* the synergistic effects of antibiotic and the alcoholic and aqueous extracts in the concentration of 90% have been study and measured for the purpose of confirm or synergy the effect of each of the antibiotic in the termination of bacteria and the extracts as antibacterial and antioxidant to the side effects that caused by the antibiotic during treatment [9]. Two mechanisms was adopted in the result, and both showed the significant effect of alcoholic green tea with lemon extract at concentration of 90% in the present of antibiotic, and this has a major role in reducing or termination of the bad side effects that comes with antibiotic when using green tea with lemon concomitant with antibiotic.

Conclusion and Recommendation

5 CONCLUSION

We conclude from this study the following:-

1- This study has shown that green tea can be further developed for use in the treatment of infectious disease.

2- This study can be utilize further in developing green tea as an alternative therapy to treat urinary tract infection caused by *E.coli.*

3- This study is pretty good in which we evaluated the activity of green tea extracts that are effective against *E.coli* isolated from clinical specimens obtained from one of the largest hospitals in Karbala

4- The synergistic action of the extracts with the antibiotics are promising by which we can reduce the harmful side effects of the antibiotics especially ciprofloxacin due to the antioxidant properties of green tea.

5- We can add a few drops of lemon juice to the green tea drink to maintenance its properties and power its effect.

6 RECOMMENDATION

1- Studying the antimicrobial effects of green tea against other infectious agents.

2- We recommended to drink green tea daily to Take advantage of its anti-microbial and antioxidant properties.

3- We recommended to add few drops of lemon juice to the green tea due to its effects in enhancing its properties.

4- Trying to purify the active ingredient in green tea and test their effect in some infections.

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