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# Study of the pathological antibacterial effects of tea extract and its role in reducing hypertension in pregnant women

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#### **ABSTRACT**

Aims: Due to the world's direction of alternative medicine and herbal medication, tea leaves have been employed to inhibit certain bacteria that cause urinary tract infections (UTIs). This study aimed to evaluate the effect of green, red and black tea as antibacterial against UTIs in pregnant women and changes in blood pressure and iron level in the blood of their women.

**Methodology and results:** Forty-eight isolates were isolated from 50 women suffering from urinary tract infections, *Staphylococcus aureus* (18) 37.5%, *Escherichia coli* (15) 31.25%, *Pseudomonas aeruginosa* (8) 16.6%, *Klebsiella* sp. (5) 10.4% and *Enterobacter* sp. (2) 4.16%. The sensitivity of bacteria to the antibiotics Amikacin, Amoxicillin/Clavulanic, Ampicillin/Sulbactam, Cefixime, Ceftriaxone, Ciprofloxacin, Imipenem, Nitrofurantion, Penicillin and Tetracycline were tested, while *E. coli* and *P. aeruginosa* (8), *Enterobacter* sp. were resistance for Ceftriaxone and Amoxicillin /Clavulanic (100%). While *Enterobacter* sp. is sensitive to Nitrofurantoin and Imipenem (100%). The ability of the isolates to form biofilms was tested using the Congo red agar method and the micro titrations plate method. The results showed that not all isolates have the ability to produce biofilms and red tea is the most powerful antibacterial under study. Drinking green tea for two weeks regularly in pregnant women who suffer from high blood pressure showed an improvement in blood pressure, as it became normal 118/78 and with the normal iron level in the blood at a rate of hemoglobin = 11.8, while drinking red tea did not change blood pressure measurements in pregnant women with high blood pressure.

**Conclusion, significance and impact of study:** The effect of red tea extract was stronger than other teas used in the study as an antibacterial against urinary tract bacteria. Regular consumed of green tea helps regulate blood pressure, especially for pregnant women who are at risk of hypertension during pregnancy.

Keywords: Antibacterial, anti-biofilm, Escherichia coli, high blood pressure, P. aeruginosa, Staphylococcus sp., tea extract

#### INTRODUCTION

The third greatest communal infection after gastrointestinal infections and respirotary tract infections in humans is (UTI). In actuality, bacterial infections in UT are the best-combined reason of both society attained contagions and nosocomial taints in hospitalized patients in the U. S. (UTIs) are cruel.

Community health difficult and are affected by a series of pathogens, however generality by *E. coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis* and *Staphylococcus saprophyticus* (Abbas *et al.*, 2020).

Green tea and other red and black teas or oolong teas are made from the *Camellia sinensis* plant, which is scientifically known as *C. sinensis* (Xing *et al.*, 2019).

Green tea plant or the so-called Chinese or Japanese tea *C. sinensis* belongs to the Theaceae family and is grown mainly in tropical and subtropical regions,

especially in Southeast Asia in Sri Lanka, India and China (Ikbal *et al.*, 2020). It is an evergreen plant in the form of small trees, with strong wedge roots.

Studies had shown that the use of green tea topically reduces the harmful effect of sunlight by neutralizing free radicals and reducing inflammation. OyetakinWhite *et al.* (2012) showed that green tea extract works to protect the skin and face from sunburn. The study showed that an infusion of about 4 mg per 2.5 cm of skin circumference protects it from sunburn. On the other hand, green tea helps to stop food toxicity by killing bacteria, in addition to the role of green tea chips in the growth of beneficial bacteria in the intestines and killing bacteria in the mouth, which helps prevent oral infections.

The Chinese used green tea as medicinal and therapeutic uses to treat cancer and considered it an antitoxicant, diuretic, aids in the elimination of phlegm, a stimulus to the stomach and aids in digestion (Ikbal *et al.*,

2020). Green tea has proven medicinal efficacy in treating amebic dysentery, bacterial dysentery and hepatitis. It is also used to treat scratches, burns, some types of cancer, colds, dog bites, dropsy, epilepsy, rashes, heat, headache and pulmonary hemorrhage (Conger and Singg, 2019).

Red tea is made from tea leaves after harvesting them and then exposing them to fermentation, which makes the tea stronger (Ikbal *et al.*, 2020). This tea contains the most amount of caffeine and it also contains antioxidant oxidation, such as a compound called epigallocatechin gallate, thearubigins, the amino acid theanine and theaflavin; which is the most prominent compound in tea and it preserves a group of compounds that are made through exposure to oxidation, between 3 to 6% of the polyphenols in tea (Chacko *et al.*, 2010).

There is no information available about the benefits of green tea for pregnant women in particular, but pregnant women are advised to avoid increasing their consumption as it does not differ from other types of tea in some characteristics, as it reduces the body's ability to absorb iron from non-animal sources. Therefore, it is preferable not to drink it during food, as it reduces the ability to absorb folic acid, which is an important nutrient to reduce the risk of spinal bifida and other neural tube defects (Butt et al., 2015).

The active chemical components of green tea is the fresh leaves of green tea contains polyphenols, saponins, caffeine, protein, pectin, fibers, starch and reduced sugars and also contains catechin, epigallocatechin and epigallocatechin gallate. It also consists of carotene,  $C_{17}H_{20}N_4O_6$ , NA,  $C_9H_{17}NO_5$  and  $C_6H_8O_6$ , the most active ingredients are caffeine and tannin (Cooper *et al.*, 2005).

In this study the effect of green, red and black tea as a microbial inhibitor against UTIs in pregnant women and changes in blood pressure and iron level in the blood of pregnant women was evaluated.

#### **MATERIALS AND METHODS**

#### **Collect isolates**

From a total of 50 urine samples of pregnant women with UTIs, 48 isolates were isolated of *P. aeruginosa, E. coli, Staphylococcus aureus, Klebsiella* sp. and *Enterobacter* sp. at Gazi Al-Hariry Educational Hospital in Baghdad during a period extended from January to April 2020.

#### **Identify** isolates

All bacterial isolates were diagnosed in the hospital laboratory, based on the morphological origins of colony coloration and bacterial growth on chromo agar medium.

The media used in this study were Mueller Hinton agar, MacConkey agar, Blood agar base (Hi media/India), Amidst CHROM agar Directive, Medium CHROM agar *S. aureus* and medium CHROM agar *Pseudomonas* (CHROM agar/France) has also been used of API-20E and API-STAPH System.

## Preparation of plants extract (Prepare aqueous plant extracts)

Some plant species were selected, including green, black and red tea and they were obtained from local markets to display their antibacterial activity on different bacterial species isolated from UTIs.

All of the purchased teas were dried and ground into a powder by an electric blender. The aqueous and cold extracts were prepared by mixing 10 g of each powder separately with 100 mL of DW in a 1000 mL volumetric flask while leaving the suspension with stirring in a water bath for 8 h and 85 °C, then filtering the products by passing through several layers of medical gauze first using Whatman No. 1 filter paper, then placing the filter in clean and sterile Petri dishes and leaving it to dry in an electric oven for 48 h or more at (45-50)µ. At that juncture, one gram of desiccated excerpts dissolve in 5 mL of DW to achieve a concentration of 100 mg/mL. Steps were followed to prepare sterile distilled water samples with 80% methanol and leave the filter material to dry in an electric oven for (1-2) h at 45-50 °C (Diriba et al., 2020).

#### **Biofilm formation assay**

All isolates were subjected to biofilm production by two methods:

#### Procedure of Congo red agar

Specific assessment of biofilm manufacturers to detect slime production was performed by using a solid culture that comprise of BHI broth 37 g/L, sucrose 50 g/L, Congo red 0.8 g/L and agar, 10 g/L. Injected dishes was incubated for 37 CO, 24 h. Slime generating strains existing black colonies, but no-creating strains developed red colonies (Abdu-Allah *et al.*, 2017).

#### Microtitre plate technique

The isolates have been incubated with a liquid medium (BHI) at 37 degrees Celsius at 18 h. Then the microbial cultures were placed in a watery medium by sterilize BHI liquid media after this the cultures have been modified in contrast to (McFarland tube number 0.5).

Two hundred microliters of the all isolates culture were added to wells of sterile polystyrene micro-titer plates (96-wells) and incubated at 48 h, 37 °C, then by with sterile normal saline the mines has been washed to eliminate unattached cells, subsequently, 200 mL of 1% crystal violet stain has been added to all well, then left at room temperature for 15 min, then it was washed four times by 200 mL sterile normal saline.

Biofilm has been produced by adding one bound of crystal violet and 200 mL of ethyl alcohol, then specified by ELISA reader at 490 nm. The 144 controls were examined using crystal violet which bonded with single visible wells culture medium lacking microbes. The microtitre-plate ODC is defined as 3 standard per version

overhead the mean OD for passive control. Strains were classified as follows: OD no attached, ODC < OD 2  $\times$  ODc softly adherent, 2  $\times$  OD < OD 4  $\times$  OD temperately attached, 4  $\times$  ODC < ODC highly adherent (Hussein *et al.*, 2018).

Biofilm inhibition spectrophotometric assay is modified by using sterile polystyrene microtiter plates (96-well) (Abdu-Allah *et al.*, 2017; Hussein *et al.*, 2018). In the same way as mentioned above after washing of the cultured microtiter plates to removal of free cells. The 25 mg/mL green tea extract was added to the wells of biofilm and incubated for 24 h at 37 °C, thereafter, washed and stained.

#### Antibacterial susceptibility test

The Kirby-Bauer method is illustrated as described by (Diriba *et al.*, 2020) re-testing of sensitivity to different antibacterial for Gram (-ve) and Gram (+ve) bacteria and a bacterial suspension prepared by (3-5) colonies from novel culture, then placed inside a tube covering 4 mL from physiological saline so that the results will be a bacterial hanging having medium cloudiness in comparison with control cloudiness liquid (McFarland Standard no. 0.5) which is approximately equal to 1.5 × 10<sup>8</sup> CFU/mL.

#### Statistical analysis

The Statistics program was used t-test to compare among groups; marked differences are significant at *p*-value <0.05.

#### **RESULTS**

Forty-eight bacterial isolates were identified as follow: *S. aureus* (18) 37.5 %, *E. coli*, (15) 31.25%, *P. aeruginosa*, (8) 16.6 %, *Klebsiella* sp. (5) 10.4 % and *Enterobacter* sp. (2) 4.16%, see Table 1.

#### Antibiotics susceptibility examination

Susceptibility of all bacteria under study toward different antibiotics was examined. Antibiotic sensitivity of five isolates of *S. aureus*, *E. coli*, *P. aeruginosa*, *Klebsiella* sp. and *Enterobacter* sp. under training to different antibiotics are shown in Table 2. From this table, *S. aureus* isolates showed an increase range of resistance in most of the antibiotics under study. The test and highest resistance antibiotics were Amoxicillin/Clavulanic, Ampicillin/Sulbactam, Cefixime and Ceftriaxone which gave resistance (100%), also *E. coli* and *P. aeruginosa* showed higher resistance to all antibiotics.

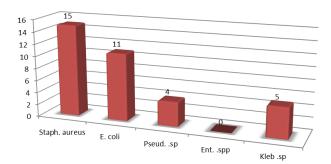


Figure 1: The isolates slim production on Congo red agar.



Figure 2: The isolates slim production on Congo red agar.

**Table 1:** Number and percentage of isolates.

Isolates	Number	Percentage %
Staphylococcus aureus	18	37.50
Escherichia coli	15	31.25
Pseudomonas aeruginosa	8	16.60
Klebsiella sp.	5	10.40
Enterobacter sp.	2	4.16
Total	48	100.00

The slime production results revealed by the red agar method are shown 15 *S. aureus* strains were positive (+) for slime production, while 3 strains were negative (-) for slime production, of which strains of highly adhesive and strains of weakly adherent, while 11 of *E. coli* isolates were positive and 4 negative (-) isolates, *Pseudomonas* sp. 4 isolates showed positive and 4 negative (11%) as well as *Klebsiella* sp. bacteria all 5 isolates are positive and of highly adherent while *Enterobacter* sp. was negative (-) for slime production, see Figure 1. Bacteria Isolates produced by biofilm, shown in blackened, reddish isolates not produced by biofilm on Congo red agar, see Figure 2.

Micro-titer plats method and ELISA device were used, which showed a very strong adhesion to *Enterococcus* and *Klebsiella* compared with *E. coli* 1, 3 and *Staphylococcus* 2 and 4 which had moderate adhesion, while the rest of the isolates recorded weak adhesion as shown in Table 3.

**Table 2:** Number and percentage of isolates resistance toward antibiotics.

Antibiotics disc	Term	Staph (18)	E. coli (15)	Pseud (8)	Kleb (5)	Entero (2)
Amikacin	AK	(11)% 61.1	(7)% 46.6	(3)% 37	(1)% 20	(1)% 100
Amoxicillin/Clavulanic	AUG	(13)% 72.2	(13)% 86.6	(5)% 62.5	(3)% 60	(2)% 100
Ampicillin/Sulbactam	MAS	(15)% 83.3	(13)% 86.6	(5)% 62.5	(2)% 40	(1)% 50
Cefixime	CFM	(14)% 77.7	(15)% 100	(8)% 100	(4)% 80	(2)% 100
Ceftriaxone	ORC	(16)% 88.8	(15)% 100	(8)% 100	(3)% 60	(2)% 100
Ciprofloxacin	CIP	(15)% 83.3	(14)% 93.3	(8)% 100	(4)% 80	(2)% 100
Imipenem	IMI	(7)% 83.8	(10)% 66.6	(5)% 62.5	(1)% 20	(0)% 0
Nitrofurantion	NT	(9)% 50	(12)% 80	(7)% 87.5	(2)% 40	(0)% 0
Penicillin	GP	(6)% 33.3	(6)% 40	(5)% 62.5	(1)% 20	(1)% 50
Tetracycline	Т	(4)% 22.2	(7)% 46.6	(6)% 75	(1)% 20	(1)% 50

Table 3: Optical density at 540 nm for all isolates formed by biofilm by Microtitre plate method.

No. isolate	Control	OD=540 nm	No. isolate	OD=Control	OD=540 nm
Staphylococcus aureus 1	0.098	0.101 <sup>N</sup>	Escherichia coli 3	0.098	0.223 <sup>M</sup>
Staphylococcus aureus 2	0.098	0.209 <sup>M</sup>	Escherichia coli 4	0.098	0.163 <sup>N</sup>
Staphylococcus aureus 3	0.098	0.090 <sup>N</sup>	Pseudomonas sp. 1	0.098	0.088 <sup>N</sup>
Staphylococcus aureus 4	0.098	$0.222^{M}$	Pseudomonas sp. 2	0.109	0.128 <sup>N</sup>
Staphylococcus aureus 5	0.098	0.158 <sup>N</sup>	Pseudomonas sp. 3	0.109	0.133 <sup>N</sup>
Staphylococcus aureus 6	0.098	0.104 <sup>N</sup>	Pseudomonas sp. 4	0.109	0.121 <sup>N</sup>
Escherichia coli 1	0.098	0.230 <sup>M</sup>	Enterobacter spp.	0.109	0.645 <sup>S</sup>
Escherichia coli 2	0.098	0.125 <sup>N</sup>	<i>Klebsiella</i> sp.	0.109	0.795 <sup>S</sup>

N=Non adherent, M=Moderate adherent, S=Strong adherent.

Table 4: Optical density at 540 nm for all isolates biofilm after treatment with of green, black and red tea extract.

No. isolate	ODs:490 nm		ODs:540 nm	
	Before treatment	After	tract	
	_	Green tea	Black tea	Red tea
Staphylococcus aureus 2	0.209 <sup>M</sup>	0.108	0.154	0.075
Staphylococcus aureus 4	0.222 <sup>M</sup>	0.106	0.144	0.067
Escherichia coli 1	0.230 <sup>M</sup>	0.119	0.127	0.102
Escherichia coli 2	0.223 <sup>M</sup>	0.123	0.144	0.098
Enterobacter spp.	0.645 <sup>S</sup>	0.225	0.234	0.122
Klebsiella sp.	0.795 <sup>S</sup>	0.098	0.299	0.185

M=Moderate adherent, S=Strong adherent.

On the other hand, the results showed that the isolates when treated with red tea extract had less growth compared to the same isolates when they were treated with green and black tea, especially *Enterobacter* and *Klebsiella* isolates, because they were stronger in biofilm production and adhesion, and as for green tea, the growth decreased compared to black tea as illustrated in Table 4.

There is very little significant variance in the control compare with treated with extract of black tea, where the control results were (0.387  $\pm$  0.262) and for the treated group (0.183  $\pm$  0.068), where t=2.545, P=0.051, as shown in Figure 3.

In addition to the presence of a slightly larger significant difference between the control and the treated isolates in green tea, treated group was (0.129  $\pm$  0.047) and t=2.531, P=0.052, as shown in Figure 4.

The results showed a difference between the control compare with the group of isolates treated in red tea extract, where it was  $(0.108 \pm 0.042)$  and t=3.045,

P=0.028. Significant differences were found between the two groups, see Figure 5.

When comparing isolates treated with the three tea extracts, black, green and red tea, red tea was found to be strongest antibacterial under study, followed by green tea and then black tea as shown in Figure 6.

Among 50 pregnant women, 19 had high blood pressure and 19 women were divided into two groups, group (A) who underwent drinking green tea regularly, while group (B) consumed red tea regularly for two weeks. On the other hand, the average of pressure measurements before drinking tea was 142/90 for groups (A) and (B) after two weeks, the blood of pregnant women for group (A) was examined. In additional to this, pregnant women who suffer from pressure problems and drank green tea regularly for two weeks, their blood pressure improved and became normal 118/78, and the iron level in the blood was very normal at a rate of hemoglobin = 11.8. While drinking red tea did not change

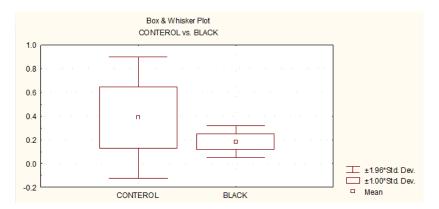
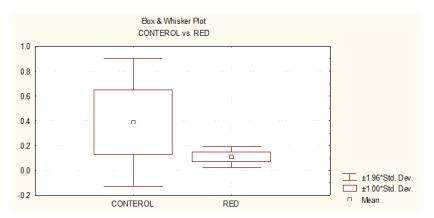
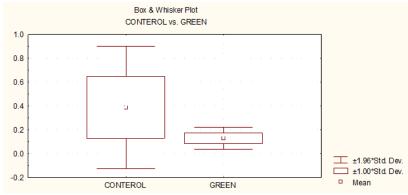


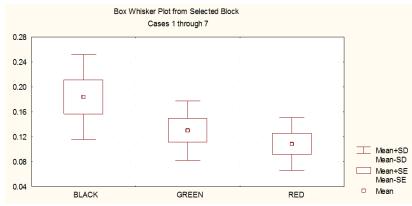
Figure 3: The difference between control and the group of isolates treated in black tea extract.



**Figure 5:** The difference between the control and the group of isolates treated in red tea extract.



**Figure 4:** The difference between the control and the group of isolates treated in green tea extract.



**Figure 6:** The comparison between the three group of isolates treated with black, green and red tea extract.

blood pressure measurements in pregnant women who suffer from high blood pressure.

#### **DISCUSSION**

Red tea has properties that reduce inflammation and danger of cancer. Through study, it was found red tea extract has a strong antibacterial compared to green and black tea, as well as green tea was stronger than black tea as an antibacterial. This due to general benefits of green tea leaves which do not oxidize, this agree with (Reygaert and Jusufi, 2013) and most of them are rich in catechins. As it outperforms other teas by 30 to 42%, especially the composite epigallocatechin-3-gallate, green tea is extra beneficial than red tea because it has antioxidants and has possessions that decrease inflammation (Yang et al., 2011), this did not match our research results, also it is a good source of vitamins, like vitamin E, C and B, which gives green tea great feature is that it contains the best main kinds of dyes, like chlorophyll and carotenoids (Yu et al., 1995). Green tea has a lesser quantity of caffeine from the red tea, where the same of 227 mL consist of 20 to 45 mg of caffeine and quantity in the tea be determined by on the preparing time besides the total of tea used in its planning (Conger and Singg, 2019).

The green tea can support lessen UTIs (Revgaert and Jusufi, 2013), where the results showed the facility of green tea extract to inhibit the growth of strains of the bacteria E. coli, which causes between 80% to 90% of UTIs, in addition to the presence of a tea compound known as epigallocatechin in the urine in high concentrations, which indicates that green tea has an antimicrobial effect. The present results agree with this reference. Green tea may contribute to relieving ulcerative colitis (Jakubczyk et al., 2020), where the study showed that the compound epigallocatechin-3-gallate in green tea has anti-inflammatory properties and reduces worsening of the condition of ulcerative colitis patients. Benefits of green tea for the stomach, it is likely safe consume by women during the periods of pregnancy and lactation, at an amount not exceeding 6 cups per day, but it is possible that it is not safe to consume larger quantities of this for a pregnant woman and this may lead to an increased risk of miscarriage and other negative effects (Chai et al., 2020). Among the different types of tea, green tea has the highest concentration of antioxidants and the reason of this is the way of manufacturing either by heated to dryness or lightly evaporated to preserve its quality and prevent the oxidation of its components, and it is worth noting that all other types of tea, with the exception of herbal teas, are all prepared by fermenting the dry leaves of the camellia plant and their different types depend on the different degree of oxidation of these leaves (Magdy et al., 2020). The present results do not agree with the results of (Laslett et al., 2020) which indicates that eating red tea causes iron malabsorption, because red tea contains antioxidants known as flavonoids and this substance may impede the absorption of iron, especially from plant

sources such as legumes and vegetables, and its richness in caffeine may also lead to a temporary rise in blood pressure, due to rapid absorption by the body for this reason pregnant women should be prevented from drinking red tea and replace it with lemon juice to iron-rich meals to increase its absorption.

#### CONCLUSION

The largest bacterial community in a urine sample of patients with UTIs is *E. coli* and *S. aureus*. The resistance of *E. coli*, *S. aureus and Enterobacter* sp. to some antibiotics is 100% and *Enterobacter* sp. is sensitive to Nitrofurantoin with Imipenem (100%). Not all isolates have the ability to produce biofilms. Red tea is the most powerful antibacterial that affects the UTI. Drinking green tea regularly improves blood pressure as well as pregnant women who suffer from pressure problems, but red tea has no effect on it.

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