Interactions of Black and Green Tea Water Extracts with Antibiotics Activity in Local Urinary Isolated *Escherichia coli*

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Abstract

A total of 17 *Escherichia coli* isolates were collected from urine specimens of patients with urinary tract infection. Antibiotics sensitivity test indicated that amikacin followed by chloramphenicol and ciprofloxacin are the most effective antibiotics. The isolates showed multidruge resistant, nine isolates were resistant to 11-15 antibiotics, 3 were resistant to 16-20 antibiotics and 5 were resistant to 21-25 antibiotic. Two isolates were selected, the first (ED1) was resistant to (22) antibiotics while the second isolate (ED2) was resistant to (14) antibiotics (out of 25). Minimum inhibitory concentration (MIC) of the black and green tea water boiled extracts were determined towards (ED1,ED2).Results showed that MIC of black tea extract was 150 mg\ml (ED1) and 100mg\ml (ED2),and the MIC of the green tea extract was 275 mg\ml (ED1) and 250mg\ml(ED2).The interaction of Sub-MIC of both extracts with antibiotics showed that black tea has synergetic effect with gentamycin, tobramycin, chloramphenicol and cefodizim, and antagonistic effect with: streptomycin, amikacin, tobramycin, kanamycin, piperacillin, cefepim, nalidixic acid, and azithromycin .Green tea has synergistic effect with : chroramphenicol, amoxicillin, azithromycin, ciprofloxacin and cefodizim and antagonistic effect with: amikacin, streptomysin, tobramycin gentamicin ,kanamycin, cefepim, azithromycin, piperacillin. This study suggests that a combination of black and green tea water extracts with some antibiotics *in vitro* has synergistic or antagonistic effect on urinary tract *E.coli* isolates.

Keywords :*Camellia sinensis* , Antibacterial , plant extracts , synergistic activity , antagonistic activity.

Introduction

The beverage known as tea is an infusion of variously processed leaves of one of the varieties of an evergreen shrub *Camellia sinensis* L. Tea is the most widely drunk beverage in the world, the green tea which is popular in the far east, differs from black tea which is familiar in the west, in that an oxidation step (called "fermentation ") occurs in the processing of the later compound but not the former one (1). 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. (2,3).

Black tea known to have a wild range of beneficial physiological and pharmacological effect, among these are, strengthening capillaries, anti-inflammatory effect, antioxidant, having hypocholestrolemic action and inhibiting the growth of implanted malignant cell (4,5).

In one of the earliest reports, an army surgeon recommended the use of tea in soldiers' water bottles as a prophylactic against typhoid. (1)

Studies on the antibacterial activity showed that extracts of tea leaves inhibits the growth of *Staphylococcus aureus* and *E.coli* which were highly sensitive (5) *S.aureus,Vibrio parahemolyticus, Clostridium perfringens, Bacillus cereus, Pleisomonas shigelloid* and *Aeromonoes sobria* failed to grow in tea normally consumed by Japanese people. Tea components also inhibit the growth of *Vibro choleras* 01, *Streptococcus mutans Shigella dysenteria, E.coli* S. *salivarius, Bordetella pertusis* and phytopathogen *Eriwinia* spp and *Pseudomonas* spp (6,7,8,9).These antimicrobial activity of tea may refere to several chemical components found in tea like polyphenolic compounds (these make up some 30% of dry weight) and generally known as "tannin" which are chemically different from other plant tannin, the simplest compound in this class are the

catechins (found in green and black tea), inhibits the growth of many bacterial species, leaves also contain flavonols, volatile flavor components (volatile oil), vitamin C, minerals and alkaloid (caffeine) (1,5,10) .

According to the reports of many researchers, antibacterial resistance is a worldwide growing problem (11) *E.coli* are facultative anaerobes in the normal intestinal flora of human and animal however pathogenic strains of these bacteria are an important cause of bacterial infections, these strains are the foremost cause of urinary tract infections as well as a major cause of neonatal meningitis, nosocomial septicemia and surgical site infections (11,12). Antibiotic therapy is the gold standard of treatment, however, long-term therapy may result in many side effects and cause selection of resistant bacteria (2).Recent reports showed that the use of plant extract may have synergic effect with some antibiotic against microorganism, Sub-MIC of black tea extract made *S. dysenteria* more susceptible to chloramphenicol ,gentamicin, methicillin and nalidixic acid and green tea have synergetic effect with levofloxacin against enteroheamorrhagic *E.coli* (6) .Epicatechin gallate (found in black and green tea) reduced the MIC of oxacillin and β-lactam in methicillin resistant *S.aureus* (MRSA) (13) and enhanced effect of Japanese tea on inhibitory activities of antibiotic against MRSA (14), so the aim of this study is to investigate the interactions of sub-MICs doses of black and green tea water extracts and 25 kinds of antibiotics against two *E.coli* strains isolated from local urinary tract infections.

Material and Methods

Bacterial isolates: A total of (17) *E.coli* isolates were collected from urine specimens submitted to a diagnostic microbiology laboratory of selected hospital (Al-Yarmook) during two months period throughout October and November 2009. The isolates were further processed by standard methods to identify as *E.coli* isolates. Isolated bacteria were maintained for daily use on Nutrient agar slants at 4 C° (12) .

Preparation of Tea Leaves

A commercial black and green trade mark variety Al-Wazah, bought from a supermarket in Baghdad, was used in the study. The leaves were grinded and the crude boiling water extracts were prepared by the method described by (15), dried powder was stored at 4 C°, and the stock solution was prepared (w/v).

Antimicrobic Sensitivity Test

The Kirby-Bauer method described by (16) was used to study antimicrobial sensitivity test. 25 antibiotic discs containing : Amikacin (10mg), Gentamicin (50mg), Streptomycin (25mg), Tobramycin (30mg), Kanamycin (30µg) Cefaclor (30mg), Cefepime (30mg), Cefodizime (30mg) Cefradin (30mg), Chloramphenicol (10µg), Vancomycin (30µg), Lincomycin (2mg), Azithromycin (15mg) Clarithromycin(15mg), Erythromycin(10µg), Amoxicillin (25mg), Ampicillin (10µg), Penicillin G (10U), Piperacillin (100µg), Ciprofloxacin (10mg), Nalidixic acid (30mg), Tetracycline (30µg), Rifampicin (30µg), Colistin (10µg), Bacitracin (10U), provided by Bioanalyse were used in this test.

Determination of Antimicrobial Activity of Black and Green Tea Water Extracts

Antibacterial activity of boiling water extracts was measured by tube test method to determine the minimum inhibitory concentration, (MICs), as described by (17, 18):

1- The concentrations 150, 125, 100, 75 mg/ml were prepared from the stock solution of black tea (200 mg/ml) and the concentrations 250, 225, 200 mg/ml from the stock solution of green tea (300mg/ml).
2- Two bacterial isolates were selected (ED1, ED2) grown on brain heart infusion (incubated at 37 C° for 24 hours).
3- Microbial suspensions were serially diluted to (10^{-3}) (containing 10^5 CFU/ml). 0.1ml were inoculated in the plant extracts concentrations (mentioned above).
4- Tubes incubated at 37 C° for 24 hours.
5- To determine the MIC, 0.1ml of each concentration inoculcated on nutrient agar plates and incubated at 37 C° for 24 hrs. the lowest antimicrobial concentration that

inhibit visible growth of bacteria is recorded as MIC (12).

Determination of Interaction Between Tea Extracts and Antibiotics

After determination of MICs for black and green tea water extracts by using tube-test method, we investigated the interaction between sub-MICs concentration and antibiotics by taking (0.1ml) from the sub-MICs concentration and spread the inoculums on nutrient agar, antibiotic discs were placed, the plates were incubated at 37C° for 24 hours.

Results and Discussions

Resistance of Isolates to Antibiotics

Antibiotics sensitivity for 17 *E.coli* isolates towards 25 antibiotics were done by measuring the diameter of inhibition zones around the discs (12, 16)

Table (1)
The Percentage of E.coli isolates resistance to 25 antibiotics.

Antibiotic	*Code*	*Concentration*	*Group*	*% for resistance*
Amikacin	AK	10mg	Aminoglycosid	0
Gentamicin	G	50mg	Aminoglycosid	41.2
Streptomycin	S	25mg	Aminoglycosid	35.3
Tobramycin	Tb	30mg	Aminoglycosid	29.4
Kanamycin	K	30µg	Aminoglycosid	41.2
Cefaclor	CEC	30mg	Cephalosporins	100
Cefepime	FEP	30mg	Cephalosporins	64.7
Cefodizime	CDZ	30mg	Cephalosporins	82.4
Cefradin	CE	30mg	Cephalosporins	100
Chloramphenicol	C	10µg	Chloramphenicols	11.8
Vancomycin	VA	30µg	Glycopeptid	100
Lincomycin	L	2mg	Lincosamids	100
Azithromycin	AZM	15mg	Macrolides	29.4
Clarithromycin	CLR	15mg	Macrolides	64.7
Erythromycin	E	10 µg	Macrolides	100
Amoxicillin	AX	25mg	β-lactam	100
Ampicillin	AMP	10 µg	β-lactam	100
Penicillin G	P	10U	β-lactam	100
Piperacillin	PRL	100 µg	β-lactam	64.7
Ciprofloxacin	Cf	10mg	Quinolones	17.6
Nalidixic acid	NA	30mg	Quinolones	41.2
Tetracycline	Te	30µg	Tetracyclins	76.5
Rifampicin	RA	30µg	Rifamycin	100
Colistin	CL	10 µg	Polymyxin	47.1
Bacitracin	B	10U	Polypeptides	100

Table (1) shows that Amikacin was the most effective antibiotic (0% resistant) followed by chloramphenicol (11.8%) and ciprofloxacin (17.6%) while cefaclor, cefradin, vancomycin, lincomycin, erythromycin, amoxicillin, ampicillin, penicillin G, refampicin and bacitracin have no antimicrobial effect towards all isolates (100%resistant). The isolates showed multidrug resistance, nine isolates(52.9%) were resistant to 11-15 different antibiotic, 3 (17.6%) were resistant to 16-20 antibiotic, 5 (29.4%) were resistant to 21-25 antibiotics, while there is no isolates resistant to 1-5 antibiotic or 6-10 antibiotic (Table 2). It have been founded that the percentage of *E.coli* isolates showing MDR was 70% in hospital patients in Brazil (19), also (20) studied acute

cystic in women reported, prevalence of multiresistant of *E.coli* of more than 20% of urine isolates to ampicillin, cephalothin and sulfamethoxazole in each year of the study, while (21) reported that isolates exhibits resistance to multiple drug classes were rare in urine samples collected during 2005-2006 in London. The prevalence of multidrug resistant *E.coli* isolates increased in the recent years specially in developing countries because most patients take antibiotics without prescriptions and sometimes use bad quality drug (5).

Table (2)
The percentage of multidrug resistant isolates.

Group	*No.antibiotic*	*No.resistant isolates*	*% of resistant isolates*
1	1-5	Non	Non
2	6-10	Non	Non
3	11-15	9	52.9
4	16-20	3	17.6
5	21-25	5	29.4

Minimum inhibitory concentration of black and green tea water extracts

Results showed that MIC of black tea water extract was 150, 100 mg/ml towards ED1, ED2 respectively, while MIC of green tea water extract was 275, 250mg/ml towards ED1, ED2 respectively. The antimicrobial activity of black and green tea studies by many researchers, (6) found that MIC of black tea extract was 88mg/ml towards *E.coli* also (2) found that the MIC of green tea extract towards 18 *E.coli* isolates was ranged from 37.5- 150mg/ml while 25mg/ml for both black and green tea extract completely inhibited *E.coli* growth after 5-7 hours (22). The methanolic extract of *Camellia sinensis* at concentration 20mg/ml was effective against *E.coli* strain (11). Also the Ethanolic extract had antibacterial effect against extended spectrum betalactamas enteric bacteria (23). *Camellia sinensis* contains many phytochemical compounds with antimicrobial properties. Tannins component (are polyphenol group) present in tea 15-20%, damage bacterial membrane, precipitate protein and having chelating properties contributed to antibacterial activity of tea (5). Catechin are simple well characterized isoflavonoides exhibit antimicrobial effect against *E.coli, V.cholera, S.mutans* and *Shigella* in vitro (24, 25).

Black tea has many more components than green tea, partly because of the oxidation processes (1), the phenolic compounds found with high concentration in black tea extract (22), that may explain our results that black tea has more antibacterial effect than green tea towards the two *E.coli* isolates.

Differences in antimicrobial activities of tea have been found to be related with kind and degree of fermentation with respect to tested bacterial strain and type of tea method of extraction (6).

Interaction of black and green tea extract with antibiotics

Sub-MICs concentration of black tea extract increased the diameter of inhibition zone (synergetic effect) of antibiotics Gentamicin (resistant to sensitive), Tobramycin (resistant to intermediate) and Chloramphenicol (intermediate to sensitive) for ED1, while it increased the diameter of inhibition zone for Cefodizime (resistant to intermediate) for ED2. Black tea decrease the diameter of inhibition zone (antagonistic effect) of Streptomycin (sensitive to intermediate) for ED1 and Amikacin, Streptomycin, Piperacillin, Cefepime, Nalidixic acid (sensitive to intermediate), Tobramycin, Azithromycin, Piperacillin (sensitive to resistant), Kanamycin (intermediate to resistant) for ED2, and the extract have no effect with Cephaclor, Vancomycin, Lincomycin, Erythromycin,

Ampicillin, PenicillinG and Bacitracin for both isolates (Table (3)).

It has been reported that Sub-bactriostatic concentration (1.5 mg/ml) of black tea have no inhibition effect with ampicillin, additive with gentamicin and tetracycline, antagonistic with streptomycin and ciprofloxacin against *E.coli* isolates (5), But (22) found that black tea has synergetic effect with amikacin that differs from our results.

Table (3)
Interaction of Antibiotics with Sub-MIC concentrations of black tea for ED1, ED2.

Antibiotic code	*Diameter of Inhibition zone "mm"*					
	ED1 control	*ED1 125mg/ml*	*ED1 100mg/ml*	*ED2 control*	*ED2 75mg/ml*	*ED2 50mg/ml*
Ak	23*	20*	20*	22*	15**	15**
G	10***	15*	10***	25*	15*	20*
S	15*	16*	12**	20*	15*	13**
Tb	12***	14**	10***	23*	12***	10***
K	5***	12***	10***	17**	10***	9***
CEC	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
FEP	NIZ	NIZ	NIZ	20*	15**	13**
CDZ	NIZ	NIZ	NIZ	NIZ	15**	15**
CE	NIZ	10***	NIZ	NIZ	NIZ	NIZ
C	16**	20*	20*	24*	20*	20*
VA	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
L	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
AZM	NIZ	NIZ	NIZ	18*	10***	10***
CLR	NIZ	NIZ	NIZ	NIZ	12***	10***
E	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
AX	NIZ	NIZ	NIZ	NIZ	6***	NIZ
AMP	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
P	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
PRL	NIZ	NIZ	NIZ	18*	13***	15**
Cf	10***	9***	10***	30*	25*	22*
NA	NIZ	NIZ	NIZ	24*	18**	17**
Te	NIZ	NIZ	NIZ	14***	14***	14***
RA	10***	10***	10***	6***	NIZ	NIZ
CL	NIZ	NIZ	NIZ	8***	NIZ	NIZ
B	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ

****sensitive , **intermediate ,***resistant ,NIZ :No Inhibition Zone***

Table (4)
Interaction of Antibiotics with Sub-MIC concentrations of green tea for ED1, ED2.

Antibiotic code	*Diameter of Inhibition zone "mm"*					
	ED1 control	*ED1 250mg/ml*	*ED1 225mg/ml*	*ED2 control*	*ED2 225mg/ml*	*ED2 200mg/ml*
Ak	23*	20*	NIZ	22*	15**	13***
G	10***	6***	NIZ	25*	10***	14***
S	15*	NIZ	NIZ	20*	10***	10***
Tb	12***	10***	10***	23*	20***	10***
K	5***	NIZ	NIZ	17**	10***	10***
CEC	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
FEP	NIZ	13***	NIZ	20*	6***	20*
CDZ	NIZ	NIZ	NIZ	NIZ	15**	16**
CE	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
C	16**	25*	NIZ	24*	20*	18*
VA	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
L	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
AZM	NIZ	15**	NIZ	18*	14**	13***
CLR	NIZ	NIZ	NIZ	NIZ	6***	6***
E	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
AX	NIZ	NIZ	14**	NIZ	6***	NIZ
AMP	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
P	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ
PRL	NIZ	10***	6***	18*	18*	15**
Cf	10***	20*	23*	30*	25*	26*
NA	NIZ	NIZ	NIZ	24*	20*	20*
Te	NIZ	NIZ	NIZ	14***	10***	10***
RA	10***	NIZ	NIZ	6***	NIZ	NIZ
CL	NIZ	NIZ	NIZ	8***	NIZ	NIZ
B	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ

****sensitive , **intermediate ,***resistant ,NIZ :No Inhibition Zone***

Table (4) shows that green tea extract has increased the diameter of inhibition zone (synergetic effect) of antibiotics Chloramphenicol (intermediate to sensitive) Amoxicillin and Azithromycin (NIZ to intermediate), Ciprofloxacin (resistant to sensitive) for ED1, Cefodizim (NIZ to intermediate) for ED2. The extract has decreased the diameter of inhibition zone (antagonistic effect) of Amikacin, Streptomycin (sensitive to NIZ) for ED1, and Amikacin, Gentamicin, Tobramycin, Streptomycin, Cefepim, Azithromycin, Piperacillin (sensitive to intermediate or

resistant), Kanamycin (intermediate to resistant) for ED2. The extract has no effect on Cefradin, Vancomycin, Lincomycin, Erythromycin, Ampicillin, Penicillin G and Bacitracin .It has been found that there is synergetic activity between soluble green tea extract and Ciprofloxacin among 93.7% of urinary tract *E.coli* isolates (2) which is close to our results. It has been shown by (26) that green tea catechins acting in synergy with beta –lactam, tetracycline and fluoroquinolons against staphylococci while (22) mentioned that green tea has synergistic effect with amikacin which is differ from our resultes .

Tea consumption coupled with its use sometimes as fluid for ingestion of orally administration drugs as well as its complex mixture of constituents suggest for interaction of tea with commonly used drugs such as antibiotics (5). There are several evidences that high levels of green tea polyphenols were found in urine after drinking tea in human and experimental animals (2), so using tea seems reasonable for treatment of UTI. Synergistic microbial growth inhibition by black tea extract and antibiotics could be attributed to the presence of dual binding sites on the bacterial surface for antibiotic and tea extract, the combined tea and antibiotics could be useful in fighting emerging drug–resistance problem (6). Catechin intercalate into phospholipids bilayers and its likely affects antibiotic resistance by perturbing the function of key processes associated with the bacterial cytoplasmic membrane (27) and made the microorganisms more susceptible to the antimicrobial agents .

Antagonistic effect may result from competing tea components with antimicrobial agent to bind to the microorganism membrane, tea component may have competitively inhibit the binding of antibiotic to the bacterial membrane and loss the activity against the bacteria in the presence of tea (5). The influence of competitive binding on the antimicrobial activity of these agents may depend on their structure this mechanism may explain the differences in the anti microbial activity of these agents in the presence of tea (28). In addition the high element content of tea (potash ,manganese ,fluorine ,aluminum and selenium) predisposes it to interaction with antimicrobial agents like tetracycline and fluoroquinolones (29 , 30).

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الخلاصة

جمعت 17 عزلة لبكتيريا *Escherichia coli* من عينات الادرار لمرضى مصابين بالتهابات المجاري البولية، وعند التحري عن حساسية العزلات لمضادات الحياة وجد ان مضاد amikacin ومن ثم chloramphenicol و ciprofloxacin كانو الاكفأ فعالية ضد ميكروبية تجاه عزلات الـ *E.coli* .اظهرت العزلات تعدد المقاومة لمضادات الحياة اذ كانت تسع عزلات مقاومة لـ 11-15 مضاد حيوي ،ثلاث مقاومة لـ 16-20 مضاد وخمس مقاومة لـ 21-25 مضاد .اختيرت عزلتان الاولى (ED1) مقاومة لـ (22) مضاد والثانية (ED2) مقاومة لـ (14) مضاد (من اصل 25مضاد) .حدد التركيز المثبط الادنى للمستخلص المائي للشاي الاسود والشاي الاخضر تجاه عزلتي البكتيريا ED2,ED1 ،وكان التركيز المثبط الادنى لمستخلص الشاي الاسود (ED1)150mg\ml و(ED2)100mg\ml اما التركيز المثبط الادنى لمستخلص الشاي الاخضر فكان (ED1)275mg/ml و250mg/ml (ED2) درست العلاقة التداخلية للتراكيز تحت التركيز المثبط الادنى للمستخلصات قيد الدراسة مع بعض مضادات الحياة . اظهرت النتائج ان مستخلص الشاي الاسود كان له فعل تازري (synergetic) مع مضادات chlomaphenicol، tobramycin، gentamicin ، ومضاد cefodizim وفعل تضادي (antagonistic) مع مضادات tobramycin ، amikacin، streptomycin piperacillin، cefepim، kanamycin azithromycin nalidixic acid ،اما مستخلص الشاي الاخضر فكان له فعل تازري مع مضادات azithromycin ، amoxicillin ، chloramphenicol ، ciprofloxacin و cefodizim وفعل تضادي مع مضادات amikacin ، streptomycin ، tobramycin، gentamicin ، kanamycin، azithromycin ، cefepim و pepiracillin . خلصت هذه الدراسة الى ان مستخلصات الشاي الاسود والشاي الاخضر لها فعالية تازرية واخرى تضادية مع بعض مضادات الحياة تجاه عزلات بكتيريا الـ *E.coli* المعزولة محليا والمسببة لالتهابات المجاري البولية خارج الجسم الحي.