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Antibacterial Activity of Camellia sinensis Extract on Uropathogenic, Extended Spectrum Beta Lactamase Producing E.coli

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ABSTRACT

The potential of antibacterial activity of Camellia sinensis extract was studied on uropathogenic Extended Spectrum Beta Lactamase (ESBL) producing *E.coli* has become the major cause of all the hospital acquired infections resulting in about 80-85% of all Urinary Tract Infections. The Multi drug resistant organisms are now difficult to treat with beta lactam antibiotics, which were used early to treat infections caused by this uropathogen. In order to avoid resistance with antibiotics we need to look for alternative treatment methods one such as; the evaluation of the antibacterial activity of Green Tea Extract which is polyphenol in nature. It contains catechin, epicatechin (EC), epigallocatechin-3-gallate (EGCg), epigallocatechin (EGC), epicatechin-3-gallate (ECG) which is responsible for greatest antimicrobial activity. Green tea is also considered as a protective agent against many types of cancers. The components exists in green tea are antioxidant and antimutagenic. The antibacterial activity of *Camellia sinensis* (Green tea) extract was tested on 10 strains of *E.coli*. The concentrations used were 110mg/ml, 130mg/ml, 150mg/ml, 170mg/ml, and 190mg/ml on agar well diffusion assay. All strains give large zones of inhibition ranging from 9-25 millimeter in diameter. The MIC of all strains ranged in between 110 to 170mg/ml, most of the strains showed MIC at 110mg/ml and at 130 mg/ml. (30%) was found susceptible at 130mg/ml, (50%) at 130mg/ml, while (10%) was found susceptible at 150mg/ml and 170mg/ml. The emergence of ESBL has been wide in centers of every different country and has become a common problem which should be resolve soon.

Keywords: *Camellia sinensis, Green Tea Extract (GTE), E.coli (Escherichia coli).*

INTRODUCTION*

The urinary tract infection is one of the common sites of most health associated infections. (Genao *et al.*, 2012). Urinary tract infections are categorized on the basis of the site of infection, the bladder (cystitis), kidney (pyelonephritis) or urine (bacteriuria), UTI can be asymptomatic or symptomatic. About 150 million cases of urinary tract infections (UTIs) occur worldwide annually. (Sharma *et al*, 2012). *Escherichia coli* are the most regular pathogens found in community acquired as well as hospital acquired urinary tract infections. (Hyle EP *et al.*, 2005). These infections can sometimes entered into blood and cause serious lethal infections such as blood poisoning disease which can be life threatening. ESBL producing organisms are very difficult to eliminate because of their multi drug resistance to various classes of antibiotics such as monobactam, carbapenems, ß-lactamase inhibitor and even to third generation cephalosporin. The most emerged mechanism of bacterial resistance among gram negatives is the production of an enzyme (in this case; penicillinase) cleaves a portion of an antibiotic and renders it inactive, Mutations can alter the receptor that transports the drugs, so the drug cannot enter the cell. Specialized membranes proteins are activated and continuously pump the drug out of the cell

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some drugs block the usual metabolic pathways, organism can circumvent this by using an alternative, unblocked pathway that produces the required product. Tea is commonly used beverage around the world. "*Camellia sinensis*" (Scientific name of Green Tea) belongs to the family Theaceae. The active polyphenols that gives the best antibacterial activity against gram positive and gram negative bacteria's are Epicatechin(EC), epicatechin-3-gallate(ECG), epigallocatechin(EGC) and epigallocatechin-3-gallate (EGCG). These polyphenols have been shown to make up 30–40% of the water-soluble solids in green tea (Carson c., Naber, 2004). The direct effects of tea component catechins are a result of its binding to bacterial lipid bilayer cell membrane which is a cause of damage in the membrane. (Sirk, *et al.*, 2009). Different studies is done on *Escherichia coli* found that when *E.coli* is exposed to the active component of a tea i.e. polyphenols. The bacterial response was regulated to 17 individual genes. Nine of 17 genes were up-regulated and eight were down-regulated. The damage to the bacterial cell membrane was the main outcomes of this change. (Cho, *et al*, 2007). The damage in bacterial cell membrane inhibits the bacterial ability binding to host cells (Sharma *et al*, 2012) this change also inhibits the bacterial binding ability of binding with each other in order to form a biofilm, which is the major cause of significant pathogenesis of infections. (Blanco, *et al* 2005). The inability of bacteria to release toxins is a result of bacterial cell membrane damage. (Sugita-Konishi, *et al* 1999; Shah, Sirk *et al.*, 2008) Bacterial lipid (phospholipid cell membranes and mycolic acid in cell walls of mycobacteria) has important function it act as an energy source; as a component phospholipid in a cell membranes of bacteria and mycolic acid in cell walls of mycobacteria) (Wang, *et al.*, 2009). It has been found that the components of the green tea (especially EGCG) inhibit specific reductases (FabG, FabI) in bacterial type II fatty acid synthesis (Zhang, 2004).

The main objective of this study was to determine the antibacterial activity of Green Tea extract against extended spectrum beta lactamase producing uropathogenic E.coli.

MATERIALS AND METHODS:

The study was conducted in a time period from August 2015 till January 2016 at Jinnah University for Women.

Study Subjects: *E.coli* isolates were obtained from culture collection of Saifee Hospital Trust. Total 10 *E.coli* isolates are used for determining Antibacterial activity of Green Tea Extract.

Preparation of Green Tea Extract: 20g powder of *camellia sinensis* were boiled in 100ml of distilled water for 10 minutes, and then filtered through whatman filter paper no 1. Now store the filtered extract in labeled sterile screw capped bottle in refrigerator for use.

Antibacterial Activity (MIC) by Agar Well Diffusion Assay: The antibacterial activity of extract of green tea was tested by agar well diffusion method. Dip the cotton swab in cultures which were previously matched and adjusted to tube no 1 0.5 standard McFarland. Remove excess culture by pressing it with the walls of the test tubes. Now make lawn on the surface of Mueller Hinton agar plates. Five wells of about 0.9mm diameter were aseptically cut on all the 10 agar-plates by using sterile borer. Five concentrations of 110mg/ml, 130mg/ml, 150mg/ml, 170mg/ml and 190mg/ml were used prepared in sterile distilled water was added to their respective wells. Incubate all the 10 plates in an incubator at 37oc for 24 hours. Next day measure zone of inhibition around each well on all plates and note down the result.

Determination of MIC: A Minimum inhibitory concentration (MIC) was determined by using Broth dilution assay method. Five concentrations (110,130,150,170,190 mg/ml) were made in distilled water with a final volume of 2 ml in each test tube for all the 10 strains of *E.coli*.

RESULTS

The results of the study showed that the leaves extract of Camellia sinensis indicates the presence of potent antibacterial activity, which confirms its use against infection. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the wells. In this study I have used different concentrations (110), (130), (150), (170), (190) mg/ml. The interpretation of results was done on the basis of resistant, intermediate, and sensitive. From (0-9mm resistant), (10-25mm sensitive) Table 1 shows clear results. The minimum Inhibitory Concentration was found on the basis of turbidity and slight color change was also observed in the test tubes in which MIC noted. The MIC value was found between 110 to 170 mg/ml. MIC of each strain is shown in the table 2 below

Figure II: Zones of inhibition against *E. Coli*

Table 1: Antibacterial Activity of green Tea Extract by Agar well Diffusion Assay

	CONCENTRATIONS				
	110mg/ml	130mg/ml	150mg/ml	170mg/ml	190mg/ml
E.coli 1	15mm	14mm	12mm	11mm	17mm
E.coli 2	20mm	13mm	17mm	18mm	17mm
E.coli 3	11mm	11mm	13mm	12mm	14mm
E.coli 4	15mm	14mm	19mm	14mm	12mm
E.coli 5	9mm	9mm	14.5mm	10mm	10mm
E.coli 6	14mm	15mm	15.5mm	13.5mm	15mm
E.coli 7	13.5mm	12mm	11.5mm	10mm	15mm
E.coli 8	13mm	13mm	12mm	12mm	14mm
E.coli 9	20mm	19mm	22mm	24mm	25mm
E.coli 10	11mm	12mm	15.5mm	12mm	13.5mm

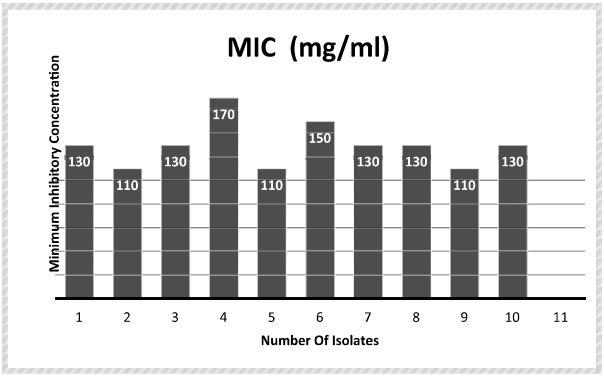

Figure 1: The minimum inhibitory concentration of different isolates

DISCUSSION

The discovery and development of antibiotics was one of the greatest advances of modern medicines. However frequent use has made pathogens resistant to that of similar antibiotic to a particular infection antibiotic and now they have acquired the characteristic of Multi Drug Resistance. To treat these infections alternative treatment methods have been evaluated to avoid their multi drug resistance traits. E.coli was found the major cause of urinary tract infections among other pathogens and about

80-85% of infections are caused by *E.coli*.

In this study Antimicrobial activity of Green Tea Extract was seen on *E.coli* 10 strain, isolated from patient's urine samples, who had symptoms of urinary tract infection, all strains were detected by the provider for positive ESBL producers. A Minimal inhibitory concentration (MIC) was determined by agar well diffusion assay in which different concentrations (110 mg/ml, 130mg/ml, 150 mg/ml, 170 mg/ml, 190 mg/ml) of Green Tea Extract were made. At 110mg/ml, 130mg/ml (90%) was found susceptible, while at 150mg/ml, 170mg/ml and at 190mg/ml the susceptibility rate was (100%) as shown in the table above in results. All the strains showed sensitivity except the fifth strain it showed resistivity at first two concentrations i;e 110mg/ml and 130mg/ml and 9mm zone diameter. (Bonjar *et al.,* 2009) evaluated the antibacterial effect of some botanical plants that were grown in the southern region of Iran against two strains of *E.coli* (PTCC No.1330 and PTCC No.1338). One of these plants was C.sinensis. Green tea had antibacterial effect against only one strain of *E.coli* (PTCC No. 1338) with 10 mm inhibition zone diameter (IZD). They suggested that green tea can be used against *E.coli* that was resistant to antibiotics such as trimethoprim and sulfamethoxazole. While the zone diameter measured was above 10mm and below 25mm which tells that e.coli is highly susceptible to Green Tea Extract.In comparative study to (Stammand Norrby, *et al.* 2001) have determine the Antimicrobial activity of the extract of Green Tea made in ethanol and the concentrations they have used are 150mg/ml, 200mg/ml and 250mg/ml concentrations and zone of inhibition noted at 150 is (27 mm), at 200 (24.1mm) and at 250 showed (18.1mm), In this study MIC of all *E.coli* isolates were found ranged in between 110 to 170mg/ml, most of the strains showed MIC at 110mg/ml and at 130 mg/ml. (30%) was found susceptible at 130mg/ml, (50%) at 130mg/ml, while (10%) was found susceptible at 150mg/ml and 170mg/ml according to (Archana. S *et al.,* 2011). They determined a Minimum Inhibitory Concentration (MIC) of *Camellia sinensis* (green tea) on *E.coli* by broth dilution method. The concentrations used were (20µl, 40µl, 60µl, 80µl, 100µl) in fresh green tea leaves he found MIC at 40ul,while in commercial green tea no MIC noted at any concentrations ,while at dust tea MIC noted at 100 ul which was the highest concentration. It means the concentration of above 100 will give better MIC'S. If comparison is to be done in between the Antibacterial activity of Green Tea Extract by agar well diffusion assay (MIC) and the Minimum Inhibitory Concentration by Broth Dilution method; It showed that well method gave better zones of inhibition on all the concentrations of Green Tea Extract as compared to broth dilution method that only gave inhibition on some (1, 2 or 3) concentrations while the concentration at which higher inhibition noted was determined as MIC.

CONCLUSION

In summary of the research conducted, it was observed that the components contained in Green tea were found effective in killing of uropathogenic ESBL producing E.coli. This has become difficult to treat with the antibiotics particularly used to cure urinary tract infections. To avoid uropathogen from multi drug resistance we need to switch to alternative treatment methods one such is use of Green tea. Green Tea is cheap, safe, and has no side effects.Green Tea has given potential results against this uropathogen.

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