



Antibacterial Activity of Medicinal Plants (Clove, Cinnamon, Garlic) Extracts and their Combined Effect with Antibiotics in Urinary Tract Infection Caused by Escherichia coli

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Abstract

Urinary tract infection is an inflammatory response of the urothelium to bacterial invasion that is usually associated with bacteriuria and pyuria. Urinary tract infections (UTI) are most familiar pathogenic infections that cause severe complications of urinary tract and difficult problems. The development of drug resistance especially to antibiotics which are used in treatment of infection is major problem. The main objective of this study was to evaluate antibacterial activity of medicinal plant extracts against pathogenic bacteria most commonly *Escherichia coli* (*E. coli*) involved in UTIs. Different bacterial strains of *E. coli* were provided by Institute of Microbiology in University of Agriculture Faisalabad. Polymerase chain reaction (PCR) was also performed for direct detection and identification of *E. coli* by targeting *UID* gene. After confirmation of *E. coli* antimicrobial sensitivity of various antibiotics as quinolones, aminoglycosides, cephalosporin and beta-lactam were tested by standard disc diffusion method. Results were recorded as by measuring diameter of zone of inhibition and analyzed by analysis of variance techniques (ANOVA). Five antibiotics ampicillin, imipenem, Ciprofloxacin, Norfloxacin and Nalidixic acid showed resistance pattern towards three strains. Clove, cinnamon, garlic aqueous and ethanolic extracts were prepared and tested to check their antibacterial efficacy against isolated *E. coli*. Clove and cinnamon extracts has showed best antibacterial activity against UTI strains as by their mean \pm SE values (13.33 ± 2.05 for clove and 11.33 ± 0.5 for cinnamon). Ethanolic extract (10%) of clove with 27 mm, boiled extract (10%) of cinnamon and garlic with 16 mm and 14 mm diameters of zone of inhibition showed maximum inhibitory response. Combined effect of plant extracts 10% with antibiotics was also tested. In combined effect, resistance drugs ampicillin, Imipenem, Ciprofloxacin, Norfloxacin and Nalidixic acid showed susceptibility pattern as by increasing diameter of zone of inhibitions on three UTI strains. Hence it is concluded that combined effect is more (additive effect) than treated UTI alone with antibiotics. Various plant extracts can be used in combination with antibiotics as a treatment therapy in *E. coli* caused UTIs.

Keywords: Urinary tract infection; *E. coli*; Antibacterial activity

Introduction

Urinary Tract Infection (UTI) is an inflammatory response of the urothelium to bacterial invasion that is usually associated with bacteriuria and pyuria [1]. It involves pyelonephritis (Kidney infection), ureteritis (ureters infection), cystitis (bladder infection) and urethritis (infections of urethra). The infectious disease UTI's have most commonly diagnosed bacterial origin, mostly involved infections are *Escherichia coli* and *Klebsiella pneumoniae*, both Gram-negative bacillus belongs to Enterobacteriaceae family [2]. About 90% of urinary tract infections are originated by pathogenic *E. coli* which infects urinary tract and known as Uro Pathogenic *Escherichia coli* (UPEC) [3].

UTI is most likely to be observed in Women than man due to anatomical differences of urinary tract. Shorter urethral opening in women facilitate transfer of pathogenic bacteria from urethral opening to inside bladder. Gastrointestinal pathogenic colonization in vaginal area has also significant role in the incidence of UTI in females [4]. In developing countries, UTI is widely spreading and difficult to eradicate because of uropathogenic bacterial persevere, deep penetration, replication within host bladder epithelial cells. Few factors responsible for UTI, bacterial colonization in tract and resulting in compromise normal host defense system are enlisted as Latrogenic/Drugs (Indwelling catheters, usage of antibiotics, Spermicides), Behavioral (voiding dysfunction, sexual intercourse), Anatomical/physiological (Vesicoureteral reflux, pregnancy), Genetic (familial tendency, susceptible uroepithelial cells, Vaginal mucus properties) [4-6].

Antibiotics belonging to quinolones, aminoglycosides, cephalosporins and beta lactam are used as treatment therapy. UTI's are so difficult to resolve because of frequent use of antibiotics which ultimately leads to development of drug resistance in pathogenic bacteria. There are three major mechanisms of resistance development which are transfer of genes known as horizontal gene transfer (plasmids, transposons and bacteriophages), incorporation of foreign DNA into bacterial DNA and formation of a new combination of genomic material and change in nucleotides sequence in chromosomes known as mutation [7]. Antibiotic-resistant of these pathogens and their complications in biological system highlight the need for alternative therapies.

Herbal medicines have been used since the beginning of human beings on this planet. The advancement of native

medicines and the use of medicinal plants carry significant financial assistances in the treatment of several ailments [8]. Natural products are preferred for the treatment of various infectious diseases because their active constitute have strong antimicrobial activity and slight adverse effects as they evaluate in case of other antibiotics. Herbal treatment highlights for new chemotherapeutic alternatives because of their strong achievements to minimize adverse drug effects, potential antimicrobial properties and reduce infections caused by drug-resistant pathogenic microorganisms [9].

Clove (*Eugenia caryophyllus*) the aromatic dried flower bud used as natural plant source of treatment in West countries and favorably in China. Clove constituents which possess various beneficial activities and antimicrobial properties are gallic ellagic and oleanoic acids, myricetin, rhamnocitrin and biflorin. Cinnamonaldehyde, eugenol, thymol and carvacrol are among essential oils extracting from clove. It has been approved from several studies that clove exhibits potent antifungal, antiviral and antibacterial effects due to its membrane functioning disruption [10]. Eugenol is principal component of clove oil showed broad antimicrobial activities against Gram-positive, Gram-negative and acid-fast bacteria, also have antifungal properties. Flavonoids in clove play an important role in various cellular defensive mechanisms such as active against various inflammatory mediators and free radical species [11].

Cinnamon (*Cinnamomum zylanicum*) has also been used in medicinal field from long time ago. Cinnamon oil contains cinnamyl acetate and alcohol and cinnamon aldehyde which show antibacterial activities. Moreover, it also contains many volatile and aromatic components which inhibit normal protein pathways. For example, cinnamon aldehyde interferes with amino acid decarboxylase normal pathway and shows antimicrobial activity [12]. Garlic (*Allium sativum*) containing organo sulfur compounds which are water soluble and are therapeutically active. Thiosulfinate are the principal constituents possessing antibiotic activity and involves in enzymatic metabolic pathways [13]. Garlic also contains numerous sulphur and phenolic substances that have powerful antifungal and antibacterial activity [14].

Keeping in view the following antimicrobial effects of medicinal plants the following project is being designed

to evaluate the combine effects of these plants with antibiotics.

Materials and Methods

Bacterial strains

Three Different bacterial strains of *E. coli* previously isolated from patients suspected to suffer from UTI was used in this study (**Figure 1**). These bacterial strains were provided by Institute of Microbiology in University of Agriculture Faisalabad.



Figure 1: Various isolated strains (*E. coli*) of UTI.

PCR identification of *E. coli*

Direct detection of *E. coli* from urinary samples was achieved by polymerase chain reaction (PCR) amplification of UID gene [15].

PCR (Polymerase Chain Reaction)

PCR is a rapid, simple and inexpensive process of producing large number of copies of DNA from minute quantities of DNA material. In 5 steps PCR process was performed. 5 steps were: DNA Extraction, Master mixer preparation, Thermal cycler process, Gel preparation and Sample loading and documentation.

Preparation of stock solution

For preservation of bacterial cultures stock solution was prepared in glycerol. Small amount of fresh culture

media from plates was inoculated in 5 ml of BHI for 6 h at 37°C. Stock solution was prepared by adding 800 µl of BHI culture broth and 200 µl glycerol and stored at -20°C.

DNA Extraction

Method for DNA extraction from bacteria

1. Bacterial culture in BHI (6 hours inoculated at 37°C) was centrifuged at 10,000 rpm for 5 min. Supernatant was discarded, added 200 µl of T-buffer and again centrifuged.
2. 700 µl of 10% SDS added to pellet and dissolved, then incubated at 65°C for 2 hours and added 700 µl phenol: chloroform: isoamyl alcohol (25:25:1), mixed by intensive shaking and centrifuged at 12,000 rpm for 4 min.
3. The upper aqueous layer was transferred carefully to a new eppendorf tube without disturbing middle layer, then Phenol: chloroform: isoamyl alcohol and centrifugation steps were repeated twice and 100 µl of 3 M sodium acetate was added to supernatant, followed by addition of 700 µl chilled isopropanol, homogenized and incubated at -20°C overnight at -70°C for 20 min.
4. Tubes were centrifuged at 12,000 rpm for 4 min and supernatant was discarded.
5. The pellet was washed with 70% ethanol and centrifuged again at 12,000 rpm for 4 min.
6. Finally, pellet was dried and dissolved in 50 µl T-buffer.

DNA was stored at 4°C until utilized [16]. Quantization of nucleic acids DNA & RNA was determined by Nanodrop spectrophotometer and conc. was found in ng/µl. 50 µl DNA solution of 50 ng conc. was formulated for each strain.

Master mixer preparation

Master mixer was prepared by mixing following constituents T-Buffer MgCl₂ dNTP Mix Forward primer Reverse primer Template DNA Taq DNA Polymerase & Water, nuclease-free. All ingredients placed in a thin-walled PCR tube and mixed all gently vortex them. PCR was performed using recommended thermal cycling conditions.

Thermal cycler

22 µl of master mixer and 3 µl of 50 ng DNA were run on thermal cycler (**Table 1** and **Figure 2**).

Detection of UID Gene

Initial denaturation temperature 94°C for 5 min 30 cycles.

Table 1: Thermal cycler process steps.

Process	Temperature (°C)	Time (min)
Denaturation	94	1
Annealing	50	1
Elongation	72	1
Final Elongation	72	5



Figure 2: Thermal cycler running of samples process for UID gene detection of *E. coli*.

Gel preparation

1.2% gel was formulated for *E. coli* sample (UID gene) by dissolving 0.72 g of agarose gel powder in 60 ml TAE Buffer (Tris-acetate-EDTA). Ethidium bromide was also added to facilitate visualization of DNA after electrophoresis (**Figure 3**). Gel was poured into a casting tray. After the gel solidified, the comb was removed, using care not to rip the bottom of the wells. The gel, still in its plastic tray, was inserted horizontally into the electrophoresis chamber and just covered with buffer.

Sample loading and documentation

Samples containing DNA mixed with loading dye (Bromophenol blue) were then pipeted into the sample wells, the lid and power leads were placed on the apparatus, and current was applied. DNA migrated

towards the positive electrode, which was usually colored red. When adequate migration occurred, the gel was placed on an ultraviolet transilluminator. After cessation of electrophoresis, photography was taken. DNA fragments were visualized by staining with ethidium bromide.

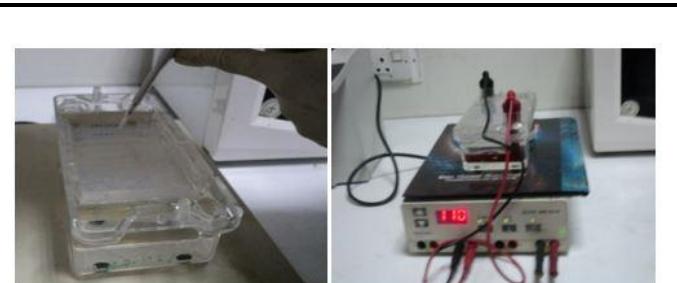


Figure 3: Sample loading in wells of gel, gel electrophoresis process.

Antimicrobial sensitivity test

Antimicrobial sensitivity tests for all isolates were checked on distinguished sensitivity test plates. Kirby Bauer method was used to check antimicrobial sensitivity. Bacterial inoculums were prepared by taking 0.5 MacFarland turbidity standards. Muller Hinton agar was prepared; autoclaved and poured in each plate. A sterile cotton culture swab was used to streak the surface of Mueller Hinton agar plates. After that plates were incubated at 37°C for 24-hour. Filter paper disks containing designated amounts of the antimicrobial drugs of OXOID® brand were used. The antimicrobial agents were Ampicillin 10 µg,

Ceftriaxone 30 µg, Norfloxacin 10 µg, Amikacin 10 µg, Nitrofurantoin 300 µg, Ceftazidime 30 µg, Cefotaxime 30 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, Nalidixic acid 30 µg and Imipenem 10 µg were tested. *Escherichia coli* ATCC25922 was taken as negative control. For DDST CTC (Cefotaxime+clavulanic acid 40 µg) and CTX (Cefotaxime 30 µg) were used and distance between two antibiotics was 20 mm. DDST was performed to test and conform strains either they were ESBL (Extended spectrum β-lactamases) or not.

Plant material

Clove (flower bud), cinnamon bark and garlic was purchased from local market of Faisalabad, identified and authenticated by the Department of Botany, University of Agriculture Faisalabad. Medicinal flower

buds bark and garlic cloves washed, dried and stored well at cool and dry place.

Clove, cinnamon and garlic aqueous extraction

- Aqueous Extracts was prepared by dissolving powdered drug (5 g) dissolved in known amount of distilled water (50 ml) and left for 24 hours at room temperature with occasional shaking. Then filtered by Whatman No.1 filter paper to obtain clear infusion. Infusion stored in sterile state before use.
- The aqueous decoction was prepared by boiling 5 g of each plant in known amount of distilled water (50 ml) in flask for 20 minutes. Then flask was removed from heat and decoction allowed to cool. Contents were filtered by Whatman No.1 filter paper to obtain clear decoction [17].

Clove, Cinnamon and Garlic Ethanolic Extraction

For ethanolic extract preparation ethanol (90%) was used as solvent. 10 g of each powdered drug was macerated in 100 ml of absolute ethanol individually and vigorously stirred with a sterile glass rod and kept overnight. Extracts were shaken in next 24 hours occasionally and the filtered through Whatman No.1 filter paper. Extraction was repeated twice, and remaining material was discarded. Filtrate was evaporated on water bath at $100 \pm 2^\circ\text{C}$ (Figure 4).

The dried extracts were sterilized by placing them under UV light for 6 hours. Each of the alcoholic extracts will reconstituted by adding 2 mL of 10% (w/v) aqueous dimethylsulfoxide (DMSO) with Tween-80 (0.5% v/v) which was sterilized prior to use by filtration through a 0.45 μm membrane filter and used for further study immediately [18].



Figure 4: Filtered and sterilized Extracts of Clove, Cinnamon and Garlic.

Preparation of sensitivity discs

Discs of 6 mm in diameter were punched out using Whatman No.1 filter paper with paper punch. After that discs were sterilized by exposing to UV light for 2 hours. Screening of antimicrobial activity of plant extracts was performed by standard disc diffusion method [16].

Sterilized disc of filter paper (6 mm diameter) was soaked in 1ml infusion (cold) on decoction (boiling) and alcoholic, for 1-2 minutes then used for screening. Zone of inhibition was measured for each extract (Figures 5 and 6).

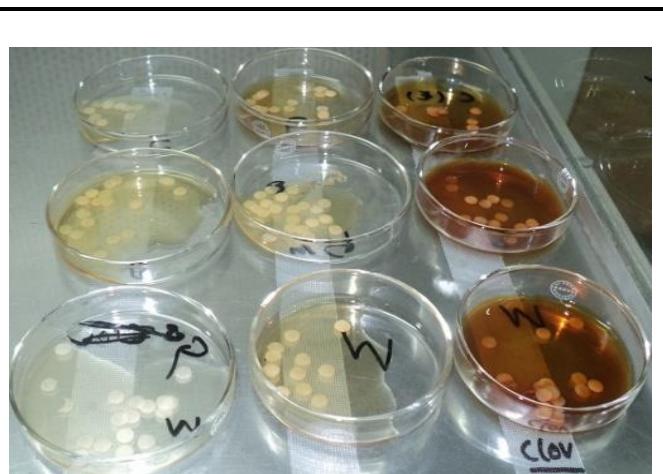


Figure 5: Discs with plants extracts.

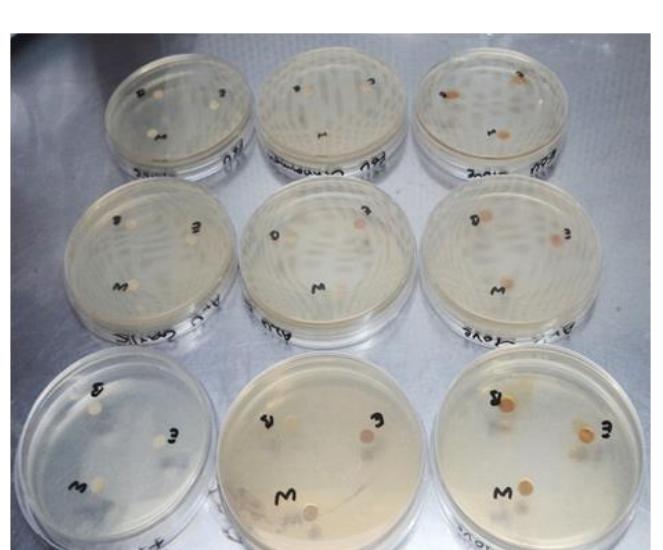


Figure 6: Plants extracts sensitivity testing against UTI strains.

Combined effect of natural plant extracts and antibiotics

Combined effect of plant extracts with antibiotics was tested by using antibiotic discs (10 drugs) in combination with prepared extracts [19]. For this purpose, total 54 media plates inoculated with stains were used, 18 plates for each strain. Results were recorded as by determination of zone of inhibition. Each antibiotic disc was soaked for 2-3 min in each plant extract and then placed on inoculated media plates (**Figures 7 and 8**).



Figure 7: 18 media plates for single strain.



Figure 8: Combined sensitivity test of plant extracts and antibiotic.

Results

Statistical analysis: The data obtained from each parameter was subjected to a statistical analysis using one-way analysis of variance techniques (ANOVA) and determined the level of significance in different parameters according to the method described by [20].

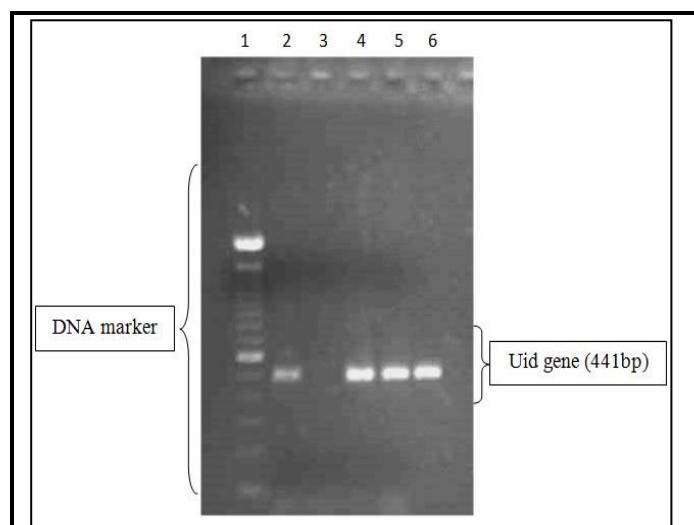


Figure 9: UID gene of *E. coli* and confirmation of strains.

PCR identification of UTI strains: In lane 1=DNA marker; lane 2=positive control; lane 3=negative control; Lane 4=strain 1; lane 5=strain 2; lane 6=strain 3.

PCR (polymerase chain reaction) was performed and UID gene in strains of 441bp product size was amplified (**Figure 9**). It was confirmed that provided strains were *E. coli* as identified by PCR.

Antimicrobial sensitivity test of antibiotics

Antibiotics sensitivity test of various drugs was performed on Nutrient agar media plates and results were recorded after 24-hour incubation. Results were recorded as by measuring diameters of zone of inhibition in mm (**Figure 10**).

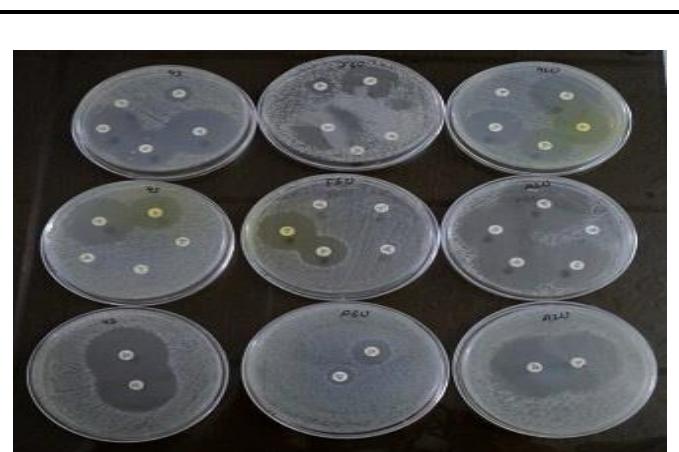


Figure 10: Antibiotics sensitivity test results.

Antibiotic groups have capital “A” letter with Mean \pm SE value in row wise and column wise comparison showed highly significant response while letter “B” showed significant activity. Antibiotic groups sharing similar letters as AB & BC have shown similar response. Antibiotic groups have capital letter “C” with Mean \pm SE value showed intermediate activity. In table, comparison of mean \pm SE of antibiotics of various groups was compared against three different bacterial (*E. coli*) strains of UTI. Ceftriaxone (CRO), Ceftazidime (CAZ), Amikacin (AK) and Nitrofurantoin (F) four antibiotics showed excellent activity against all strains as shown by capital letter “A”. While Imipenem (IPM) and Gentamicin (CN) has showed good activity against three strains as denoted by capital letter “AB”.

Ciprofloxacin (CIP) and Norfloxacin (NOR) has showed least activity against three strains and these fluoroquinolones showed pattern of resistance on *E. coli* isolates of UTI. Nalidixic acid (NA) and Ampicillin (AMP) has showed resistance against three strains. Ampicillin (AMP) and Nalidixic acid (NA) showed maximum resistance towards all strains while third generation cephalosporin's also exhibit good response against all strains. In vertical comparison of mean \pm SE among three strains, strain 1 showed highly significant activity while strain 2 and strain 3 has showed similar response. Strain 1 was effective against mostly tested antibiotics while strain 2 and strain 3 were MDR (Multidrug resistance) strains, showed resistance to various groups of antibiotics (Table 2).

Table 2: Statistical analysis of results.

Groups	ZONE			Mean
	Strain 1	Strain 2	Strain 3	
AMP	6	6	6	6.00 \pm 0.00 C
CRO	40	14	35	29.67 \pm 7.97 A
CAZ	34	20	32	28.67 \pm 4.37 A
IPM	36	6	33	25.00 \pm 9.54 AB
CN	30	31	13	24.67 \pm 5.84 AB
AK	31	27	26	28.00 \pm 1.53 A
CIP	26	6	6	12.67 \pm 6.67 BC
NOR	23	6	6	12.00 \pm 5.51 BC
NA	6	6	6	6.00 \pm 0.00 C
F	30	26	23	26.33 \pm 2.03 A
Mean	26.20 \pm 3.69 A	14.90 \pm 3.22 B	18.60 \pm 3.94 B	

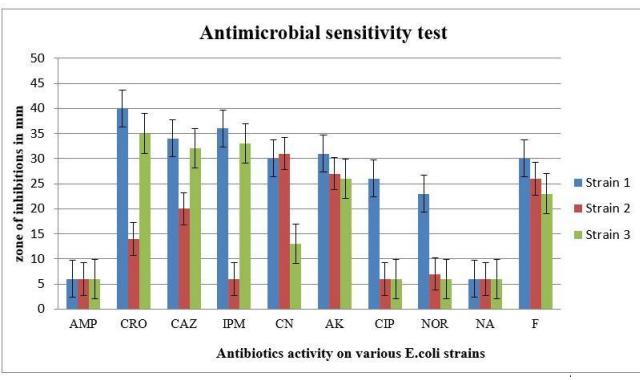


Figure 11: Antibiotics activity on various *E. coli* strains.

In case of strain 1, ampicillin and Nalidixic acid showed resistant against all three tested *E. coli* strains of UTI. In strain 2, five antibiotics ampicillin, Imipenem, Ciprofloxacin, Norfloxacin and Nalidixic acid were resistant hence it was a MDR (multidrug resistance strain). In case of strain 3, four antibiotics ampicillin, Ciprofloxacin, Norfloxacin and Nalidixic acid showed resistance against three tested strains. It was also a MDR (multidrug resistance strain) (Figure 11).

Double disc synergistic test (DDST)

Double disc synergistic test was performed on three strains of UTI source to confirm either these three

strains of UTI source were ESBL (Extended spectrum β -lactamases) or not. Here CTC (Cefotaxime+Clavolanic acid) double disc was used and CTX (Cefotaxime) as a single disc. Distance between two discs was 20 mm. Results were observed after 24-hour incubation of media plates (**Figure 12** and **Table 3**).



Figure 12: DDST (Double disc synergistic test) to confirm ESBL strains.

Table 3: DDST (Double disc synergistic test) to confirm ESBL strains.

Strains	Zone of inhibition in mm		Results
	CTC (Cefotaxime+ Clavolanic acid)	CTX (Cefotaxime)	
strain 1	37	40	NO ESBL
strain 2	16	11	ESBL
strain 3	30	36	NO ESBL

According to CLSI guidelines when difference of diameters of zone of inhibition between double disc and

single disc is ≥ 5 they are confirmed ESBL (Extended spectrum β -lactamases). Here in results, one strain as strain 2 was ESBL while other two strains were non ESBL as indicated by difference of their diameters of zone of inhibition of double disc and single disc. Due to ESBL enzyme production strain 2 was multi drug resistance strain as indicated above in results.

Antibacterial effect of various plants extracts:

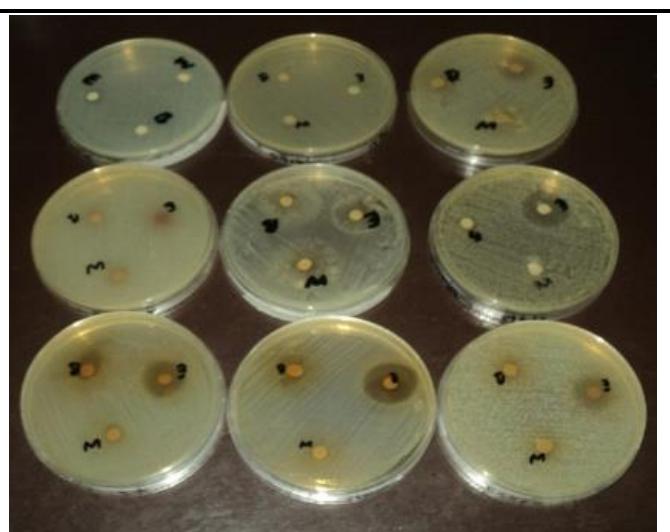


Figure 13: Various plants extracts sensitivity test on isolates strains of UTI.

Antimicrobial sensitivity test of various plants extracts was performed on Nutrient agar media plates inoculated with three strains of UTI and results were recorded after 24-hour incubation (**Figure 13**). Results were recorded as by measuring diameters of zone of inhibition in mm. results were tabulated as (**Table 4**):

Table 4: Plants extracts sensitivity diameters of zone of inhibition in mm.

Plants		Zones in mm of various <i>E. coli</i> strains		
	Extracts	Strain 1	Strain 2	Strain 3
Clove	Water	15	11	10
	Boil	7	8	10
	Ethanol	15	27	17
Cinnamon	Water	10	11	9
	Boil	14	16	14
	Ethanol	12	13	10
Garlic	Water	6	9	9
	Boil	10	14	12
	Ethanol	9	13	11

Plants extracts sensitivity was tested and diameter of zone of inhibition was measured for every plant extract such as aqueous, aqueous boil and ethanol extracts of clove, cinnamon and garlic. Ethanolic extract of clove showed best activity among three extracts of clove. Aqueous extract of clove also possessed antibacterial activity as indicated by their zone of inhibitions. Cinnamon boil extract was also showed potential antibacterial effect against three strains of UTI.

Ethanolic extract of cinnamon showed antibiotic effect on UTI strains. Garlic aqueous and boiled extracts gave almost similar antibacterial activity against tested *E. coli* isolates. Maximum zone of inhibition was recorded as 27 mm of clove ethanolic extract against strain 2 which was ESBL and MDR also. In cinnamon extracts boiled extract maximum effect was 16mm recorded by strain 2. Overall these plants clove, cinnamon and garlic extracts (Aqueous, boil and ethanol) except of garlic aqueous extract on three strains showed best antibacterial response as indicated by their diameter of zone of inhibition in mm (**Figure 14**).

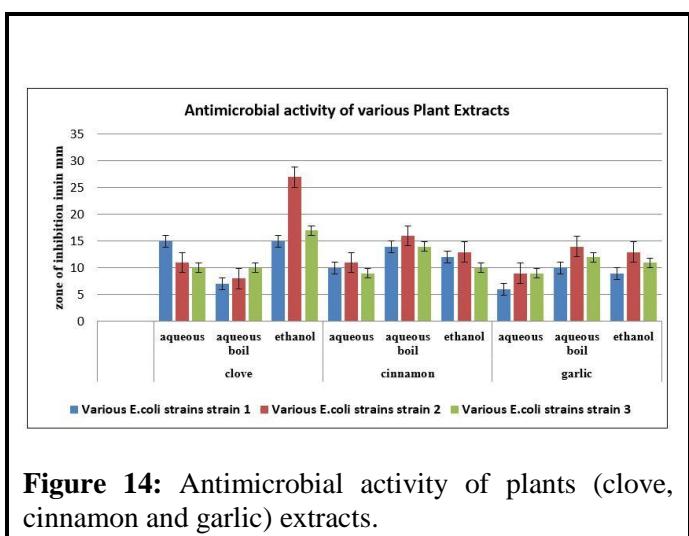


Figure 14: Antimicrobial activity of plants (clove, cinnamon and garlic) extracts.

Statistical analysis of results:

Various strains (strain 1, strain 2 and strain 3) and plant treatments (clove, cinnamon and garlic) observed in combination. Comparison of antibacterial effect of three strains with each other and various plants antibacterial effect is compared with others.

Plants extracts activity was also checked on these strains. In Table 5 UTI Strains × Treatment of mean ± SE of plants extracts) three strains were treated with three different extracts (aqueous, boil, ethanol) of clove cinnamon and garlic. Plants extracts showed good

antibacterial activity against strains as indicated by their mean ± SE value (**Table 5**).

Table 5: UTI Strains × Treatment of mean ± SE of plants extracts, A=Highly significant response.

Treatment	Strain		
	Strain 1	Strain 2	Strain 3
Aqueous	10.33 ± 2.6	10.33 ± 0.67	9 ± 0.58
Aqueous boil	8 ± 1.53	9 ± 1.53	10.67 ± 1.76
Ethanol	12 ± 1.73	17.67 ± 4.67	12.67 ± 2.19
Mean	10.11 ± 1.16 A	12.33 ± 1.96 A	10.78 ± 0.98 A

Various plants clove, cinnamon and garlic in comparison with their various extracts such as aqueous, boil and ethanol extracts. Extracts were in comparison with each other. Ethanol extract of clove as indicated by small letter “a” and boil extracts of cinnamon denoted by “b” and garlic represented by “d” showed potential antibacterial response among all extracts while in overall comparison of three plants clove, cinnamon and garlic first two plants clove and cinnamon extracts as by their mean values ± SE (13.33 ± 2.05 A for clove and 11.33 ± 0.53 A for cinnamon) has showed best antibacterial activity than garlic extracts against UTI strains. Ethanolic extract of clove is more effective as compared to boil and water extracts against isolated *E. coli* (**Table 6**).

Table 6: Plants extracts treatments in comparison of mean ± SE, Small letters (a, b, c, d) represent comparison among interaction means and capital letters are used for overall mean.

Treatment	Plant		
	Clove	Cinnamon	Garlic
Aqueous	12 ± 1.53 bc	10 ± 0.58 bcd	7.67 ± 0.88 cd
Aqueous boil	8.33 ± 0.88 bcd	12.33 ± 0.88 b	7 ± 0.58 d
Ethanol	19.67 ± 3.71 a	11.67 ± 0.88 bc	11 ± 1.15 bcd
Total	13.33 ± 2.05 A	11.33 ± 0.53 A	8.56 ± 0.77 B

Table 7: Plants extracts Treatment on three Strains with mean \pm SE, A=Highly significant, B=Significant.

Strain	Treatment		
	Aqueous	Aqueous boil	Ethanol
Strain 1	10.33 \pm 2.6	8 \pm 1.53	12 \pm 1.73
Strain 2	10.33 \pm 0.67	9 \pm 1.53	17.67 \pm 4.67
Strain 3	9 \pm 0.58	10.67 \pm 1.76	12.67 \pm 2.19
Total	9.89 \pm 0.82 B	9.22 \pm 0.89 B	14.11 \pm 1.81 A

Various plants extracts (aqueous, boil and ethanol) activity compared against three different strains. Three different strains treated with various plants extracts. Aqueous and aqueous boiled extracts of plants showed similar significant antibacterial response as indicated by their mean \pm SE values while ethanolic extracts showed highly significant antimicrobial activity against all strains of UTI used in this study (**Table 7**).

Combined effect of plants extracts and antibiotics

Combined effect of plants (clove cinnamon and garlic) extracts (aqueous, boil and ethanol) and antibiotics were also performed. Prepared antibiotic discs loaded with specific amount of drug were soaked in each plant extract separately for 2-3 min and then combined sensitivity test was performed by disc diffusion method on inoculated media plates with UTI strains. Plates were incubated for 24 hours and results were recorded as by measuring diameters of inhibitory zones formed in circles around each drug (**Figure 15**).

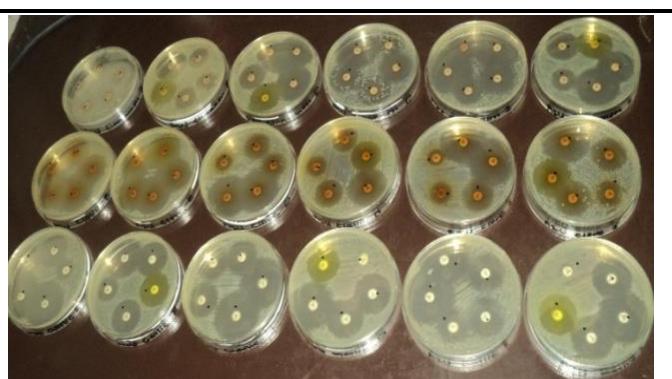


Figure 15: Combined effect of plants Extracts and antibiotics on strain 1.

Diameters of zone of inhibition was measured in mm for each drug with every extract (aqueous, boil and ethanol) of plants as clove, cinnamon and garlic.

In case of strain 1, ampicillin and Nalidixic acid were resistant but in combined therapy their raised diameters of zone of inhibition indicated that combined effect is increased, and resistance changed into susceptibility (**Figure 16**).

Antimicrobial discs	E COLI strain 1										
	antibiotic effect	clove		cinnamon		garlic					
		aqueous	boil	ethanol	aqueous	boil	ethanol	aqueous			
Ampicillin (Amp)		6	16	16	31	19	26	16	9	6	8
Nalidixic acid (NA)		6	13	15	32	23	28	17	21	21	18

Figure 16: Combined effect resistance to susceptibility pattern on strain 1.

In case of strain 2 which is ESBL and MDR (multi drug resistance) combined effect was also more than antibiotics alone effect and resistance was reduced.

Here five antibiotics ampicillin, Imipenem, Ciprofloxacin, Norfloxacin and Nalidixic acid were resistant but in combined therapy showed susceptibility (**Figure 17**).

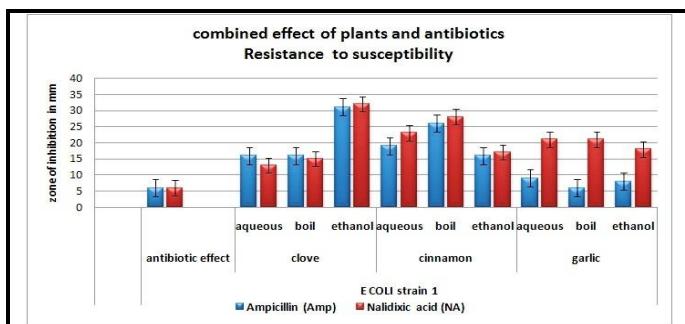


Figure 17: Combined effect resistance to susceptibility pattern on strain 1.

In strain 3 which was also MDR four antibiotics showed resistance alone but in combination with extracts zone of inhibition was increased for each drug (**Figures 18-23**).

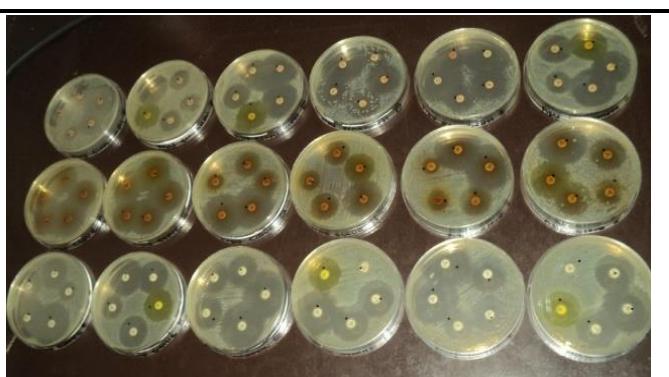


Figure 18: Combined effect of plants Extracts and antibiotics on strain 2.



Figure 21: Combined effect of plants Extracts and antibiotics on strain 3.

Antimicrobial discs	E COLI strain 2									
	antibiotic effect	clove			cinnamon			garlic		
		aqueous	boil	ethanol	aqueous	boil	ethanol	aqueous	boil	ethanol
Ampicillin (Amp)	6	16	18	28	17	22	21	18	18	19
Imipenem (IPM)	6	13	15	32	23	28	17	21	21	18
ciprofloxacin (CIP)	6	27	24	31	29	30	22	26	28	28
Norfloxacin (NOR)	6	25	22	31	27	24	21	27	26	27
Nalidixic acid (NA)	6	13	15	32	23	28	17	21	21	18

Figure 19: Combined effect resistance to susceptibility pattern on strain 2.

Antimicrobial discs	E COLI strain 3									
	antibiotic effect	clove			cinnamon			garlic		
		aqueous	boil	ethanol	aqueous	boil	ethanol	aqueous	boil	ethanol
Ampicillin (Amp)	6	16	17	26	19	22	16	18	18	10
ciprofloxacin (CIP)	6	16	16	21	16	22	20	17	17	18
Norfloxacin (NOR)	6	15	16	24	17	22	18	18	17	19
Nalidixic acid (NA)	6	19	15	21	17	20	17	18	20	21

Figure 22: Combined effect resistance to susceptibility pattern on strain 3.

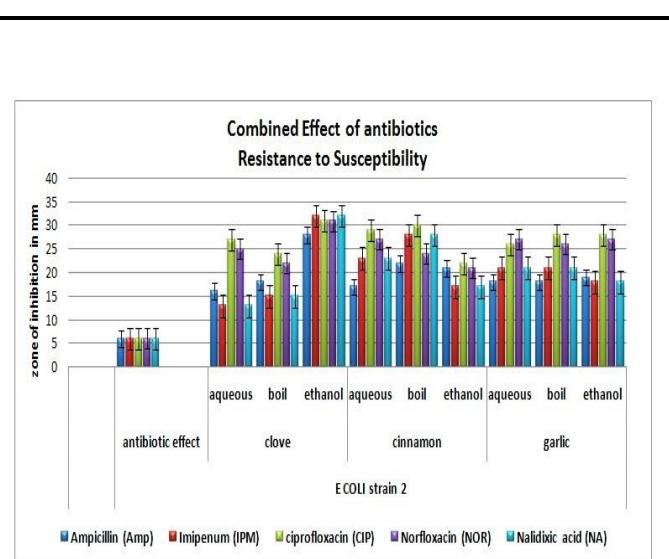


Figure 20: Combined effect resistance to susceptibility pattern on strain 2.

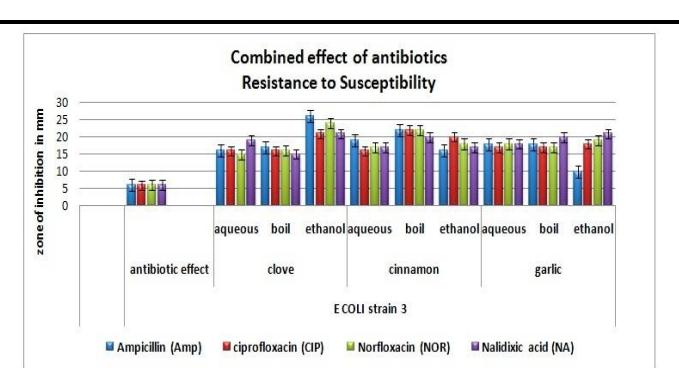


Figure 23: Combined effect resistance to susceptibility pattern on strain 3.

Discussion and Conclusion

PCR was performed and confirmed that provided UTI strains belongs to *E. coli*. Then antibiotic sensitivity test, plants extracts sensitivity test and combined effect

of plants extracts and antibiotics against three *E. coli* strains were performed. Results were recorded as by measuring diameters of zone of inhibition of various antibiotics, plants extract, and combined effect of plants extracts and antibiotics. DDST was also performed. According to CLSI guidelines when difference of diameters of zone of inhibition between double disc and single disc is ≥ 5 they are confirmed ESBL. Here in results, one strain was ESBL while other two strains were non ESBL as indicated by difference of their diameters of zone of inhibition of double disc and single disc.

In Table 2 comparison of mean \pm SE of antibiotics of various groups were compared against three different bacterial (*E. coli*) strains of UTI. Ceftriaxone (CRO), Ceftazidime (CAZ), Amikacin (AK) and Nitrofurantoin (F) four antibiotics showed excellent activity against all strains as shown by capital letter "A". While Imipenem (IPM) and Gentamicin (CN) has showed good activity against three strains as denoted by capital letter "AB". Ciprofloxacin (CIP) and Norfloxacin (NOR) has showed least activity against three strains and these fluoroquinolones showed similar pattern of resistance on *E. coli* isolates of UTI as given by Vander et al. Nalidixic acid (NA) and Ampicillin (AMP) has showed resistance against three strains as reported in Shafique et al. Resistance pattern was similar as reported by Guidoni et al. such as Ampicillin (AMP) and Nalidixic acid (NA) showed maximum resistance towards all strains while third generation cephalosporin's also exhibit good response against all strains. In vertical comparison of mean \pm SE among three strains, strain 1 showed highly significant activity while strain 2 and strain 3 has showed similar response. Strain 1 was effective against mostly tested antibiotics while strain 2 and strain 3 were MDR (Multidrug resistance) strains, showed resistance to various groups of antibiotics as reported by Bashir et al.

Plants extracts activity was also checked on these strains. In Table 6, three strains were treated with three different extracts (aqueous, boil, ethanol) of clove cinnamon and garlic. Plants extracts showed good antibacterial activity against strains as indicated by their mean \pm SE value.

In Table 6, various extracts were in comparison with each other. Ethanol extract of clove and boil extracts of cinnamon and garlic showed excellent activity among all extracts while in overall comparison of three plants clove, cinnamon and garlic first two plants clove and cinnamon extracts has showed best antibacterial activity

than garlic extracts against UTI strains. Ethanolic extract of clove is more effective as compared to boil and water extracts against isolated *E. coli*. Results are in accordance as obtained by [21]. The results illustrated that there is synergism between the combination of alcoholic extract of clove and antibiotics (Amikacin, gentamicin, levofloxacin and norfloxacin). Cinnamon aldehyde was a major component of cinnamon bark and showed antibacterial activity on urinary pathogens *E. coli*. Results were in agreement with the previous reports [22].

Various garlic extracts were also prepared in water, boiled extract and in ethanol. Boiled and ethanol extracts showed inhibitory effect on selected isolates. Antibacterial effect of garlic was due to presence of thiosulfinate, the principal constituents possessing antibiotic activity [23]. Results were in accordance to [24].

In Table 4, three strains treated with different plant extracts and among all ethanolic extracts of clove cinnamon and garlic have greater mean \pm SE value indicated a strong antimicrobial and antibacterial potential. Aqueous and aqueous boil extracts showed similar activity. Various treatments of extracts against bacterial strains have an excellent antibiotic effect as observed by their mean \pm SE values. So, it is concluded that clove and cinnamon plants extracts can be used as treatment against urinary tract infections caused by *E. coli*.

In combined effect, plants extract used in combination with various antibiotic groups and their combined effect was observed. In combination with plants extracts antibacterial effect of various antibiotics enhanced. Zone of inhibition of antibiotics increased with extracts against UTI strains. Some antibiotic groups which have showed resistance against strain 1, strain 2 and strain 3 when used in combination with plants extracts their diameter of zone of inhibition were increased. Plants extracts showed additive effect with antibiotics. Plant could be considered as a source of compounds that increase the sensitivity of bacterial cells to antibiotics [25].

In case of strain 1, ampicillin and Nalidixic acid were resistant but in combined therapy their raised diameters of zone of inhibition indicated that combined effect is increased and resistance changed into susceptibility [26].

In case of strain 2 which is ESBL and MDR (multi drug resistance) combined effect was also more than antibiotics alone effect and resistance was reduced. Here five antibiotics ampicillin, Imipenem, Ciprofloxacin, Norfloxacin and Nalidixic acid were resistant but in combined therapy showed susceptibility [27-29].

Hence it is concluded that combined effect is more than treated UTI alone with antibiotics. Various plant extracts can be used in combination with antibiotics as a treatment therapy in *E. coli* caused urinary tract infections.

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Conflict of Interest

The authors have no actual or potential conflicts of interest to report.

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