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EFFICACY OF MEDICINAL PLANT EXTRACTS AND ANTIBIOTIC COCKTAIL TO ABATE MULTIDRUG RESISTANT BACTERIA FROM URINARY TRACT INFECTIONS

Ahsan Naveed[1], Sabahat Abdullah[1], Dr. Rakhad Alrawi[2]*, Rabia Naveed[3] and Muhammad Ammar Naveed[4]

[1]Institute of Microbiology, Department of chemistry and biochemistry. University of Agriculture, Faisalabad, 3840, Pakistan.

[2]Grand Canyon University, Arizona, USA.

[3]Department of chemistry and biochemistry. University of Agriculture, Faisalabad, 3840, Pakistan.

[4]Institute of Dentistry, CMH Lahore Medical and Dental College, Lahore, Pakistan.

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***Corresponding Author**

Dr. Rakhad Alrawi

Grand Canyon University, Arizona, USA.

ragad.rawai@googlemail.com

ahsannaveed.uaf@gmail.com,

sabahatabdullah41@gmail.com,

ABSTRACT

The Enterobacteriaceae family produces extended spectrum β-lactamases enzymes in response to β-lactam antibiotics. These enzymes have the potential to hydrolyze the antibiotics like penicillin, cephalosporin and monobactam except the carbapenem. The present study was designed to evaluate the antibacterial activity of specific plant extracts alone and in combination with some of the antibiotics routinely used against the *E.coli*. A total of six extracts of three plants namely *Zingiber officinale* (ginger), *Piper nigrum* (black pepper) and *Thymus vulgaris* (thyme) in two different solvents (ethanol and water) were evaluated for their antibacterial activity against three bacterial strains of *E.coli* from different nosocomial origin. The antibacterial activity was measured by Kirby-Bauer disc diffusion method and detection of ESBL *E.coli* was done using double-disc synergy method and then by PCR. The results of present study revealed that all bacterial isolates under study were resistant to more than 3 classes of antibiotics. While these resistant strain showed susceptibility to plant extracts when given in combination with antibiotics. It was concluded that plant's extracts have the potential to increase antibacterial properties of antibiotics when used as cocktail.

KEYWORDS: Medicinal plant, Antibiotics, Antibacterial activity, multidrug resistant bacteria.

INTRODUCTION

Escherichia coli (*E.coli)* is a Gram negative, anaerobic and oxidase negative organism. It causes Gram negative sepsis, urinary tract infections, gastroenteritis, *E.coli* associated diarrheal disease, meningitis in neonates, wound infections, endotoxin induced shock, and pneumonia in immunocompromised hospitalized patients.[1] Gram negative organisms are one of the sources of hospital acquired infections. *E.coli* have wide range of virulence factors such as capsule, antigenic phase variation resistant to serum killing, endotoxin, sequestration of growth factors and antimicrobial resistant. Antibiotics are chemical agents which can inhibit bacterial growth. Now-a-days the resistance to antibiotics is an emerging issue to human. Antimicrobial drugs are capable to treat and inhibit several infectious diseases. Due to increasing human population there is a huge rise in antibiotic use, which resulting in resistance of bacteria to antibiotics.[2] Extended spectrum beta lactamases (ESBLs) due to the production of β-lactamase enzymes are capable of hydrolyzing β-lactam antibiotics such as aztreonam, cefotaxime, penicillins, and ceftriaxone.[3,4] The previous ESBLs were derived from TEM-1 and SHV-1 but now CTX-M is a modern type of ESBL.[3,5,6,] CTX-M has a potential to hydrolyze cefotaxime. This property of CTX-M is causing nosocomial infections. Resistance against antimicrobial agents is an issue that should be consider as it is a reason for high mortality among individuals.[7,8] The inappropriate use of antimicrobial agents is one the cause of resistance.[9] There are four ways by which bacteria cause resistance to antibiotics including enzymatic activity, extrusion by efflux, reduction in cellular uptake and modification at the site.[10,11] The use of herbs had been considered since ancient times. Pakistan is rich country in many medicinal plants that can be used for treating ailments. Different portions of plants vary with their chemical properties. The chemical components are extracted from aerial and root parts of plants and were used by researchers in medicinal field with surprising effects. Among the plants are: **Black pepper**, is declared as the king of spices.[12] The crude extract of this spice is Piperine that has potential to kill bacteria. **Ginger**, the chemical components constitute gingerdiol, gingerol, gingerone and gingerdione. These components are best against enterotoxic *E.coli*.[13,14] **Thyme**, also termed as zaitar, is effective against *E.coli* due to the chemical thymol and carvacrol. The plant extracts can control the

mechanism of resistance of bacteria when incorporated with antibiotics.[15]

The objective of this study is to investigate the effects of black pepper, ginger and thyme as possible antibacterial medicinal plants against *E.coli* and their combined effect with different antibiotics.[16,17]

MATERIALS AND METHODS

Three bacterial strains of ESBL producing *E.coli* isolates were Gram stained and inoculated on MacConkey agar plates, overnight at 37°C±1°C in an incubator, their zone of inhibition was observed. A bacterial inoculum according to 0.5 McFarland standards was prepared. Plates containing Mueller-Hinton medium were inoculated with prepared bacterial inoculum. ESBL was detected by phenotypic and by double disc synergy test. For genotypic characterization polymerase chain reaction was employed. According to CLSI guidelines, for ESBL *E.coli* identification, disc diffusion method was used. The lawn culture of bacterial isolates was prepared and the resistance pattern was tested against Ceftazidime (30µg), Ceftriaxone (30µg) and Cefotaxime (30µg) after an incubation for 24h at 37°C. Their zone of inhibitions was measured. The strains showing zone diameter ≤22mm for Ceftazidime, ≤25mm for Ceftriaxone and ≤27mm for Cefotaxime were selected as ESBL producer as recommended by CLSI. The strains that resist at least one of the screening agents were further examined for ESBL production. The 30µg disc of Cefotaxime was placed on the 1st half of culture plate at a distance of 2cm from center to center cefotaxime/clavulanic acid to other half of the plate. Overnight incubation of plate at 37°C was given and their zone of inhibition was measured. Following the zone measurements, the diameter of zone of inhibition around Cefotaxime disc alone was subtracted from diameter of zone of inhibition around Cefotaxime/Clavulanic acid. The difference of ≥5mm was considered to isolate as ESBL positive. Polymerase chain reaction (PCR) applied to extract and amplified plasmid DNA using phenol-chloroform, PCR master mix of buffer, dNTPs, primers and Taq DNA polymerase 0.22µL master mix + 3µL DNA (50ng) and placed in PCR thermal cycler. PCR was then performed using recommended thermal cycling conditions. PCR products were analyzed by the agarose gel electrophoresis. For determination of co-resistance among all bacterial isolates, Kirby-Bauer disc diffusion method was used. Different antibiotics (Ciprofloxacin, Amikacin, Gentamicin, Nalidixic acid, Norfloxacin, Ceftazidime, Ampicillin, Imipenem, Nitrofurantoin, and Ceftriaxone) were used to check multi-drug resistance by ESBL *E.coli*. Black pepper berries, ginger rhizome and thyme leaves were purchased from local market and authenticated by department of Botany, University of Agriculture, Faisalabad. Plants were washed thoroughly with distilled water to remove dust and any other extraneous material and air dried. Black pepper berries and thyme leaves after cleaning were crushed into fine powder. Ginger's outer covering was peeled off and cut into slices. These slices were dried in hot air oven at 65°C for 48 hours and crushed into powder form. For ethanolic extraction of black pepper, thyme and ginger, 20gm of black pepper and thyme powder were soaked into 60ml of 95% ethanol for one day. For ginger extraction, 5gm of ginger powder was added to 200ml of 95% ethanol and stir vigorously to dissolve it. Then material was filtered. For aqueous extraction, 300ml of sterile distilled water was added to the 30gm of ground dried black pepper and thyme, heated below boiling point and stirred for 2½-3hour. For aqueous extraction of ginger, 5gm of ginger powder was added to 200ml distilled water and stir vigorously to dissolve it. After 24hour interval, mixture was filtered and the precipitates were discarded and supernatant was collected for evaluation. Whatmann filter paper #1 was punched out with paper borer and disc of 6mm were prepared and subjected to autoclave in order to sterilize the prepared discs. Then these discs were soaked in 1ml aqueous and ethanolic extracts separately for 1-2 minutes then used for screening after being dried. Sterility was checked by streaking the extracts on nutrient agar medium and incubated at 37°C for 24 hours. The no growth on themedia was considered for the extract to be sterile. Antibiotic discs dipped in plant extracts were used to evaluate its combined antibacterial activity. Sterilized discs of 6mm diameter were placed sterilized conditions in various extracts for about one minute then fixed on Mueller-Hinton plates inoculated previously by bacterial suspension. After that all cultured plates were placed in an incubator at 37±2°C for 18-24 hours; after that area of inhibition was observed and measured in millimeter (mm). Plants extracts were extracted using solvents ethanol 80% and deionized water. To check the combined effect of natural plants extract and antibiotics, the pre-prepared antibiotic discs were soaked into plant extract for about 1-2minutes and then used for screening. These discs were then fixed on the Mueller-Hinton agar plate inoculated with bacterial inoculum and incubated at 37°C for 24 hours. Then their diameter of zone of inhibitions were observed and measured.

RESULTS

In the Gram staining method for detection of bacteria, *E.coli* was confirmed by pink colonies on MaConkey agar. In phenotypic screening of ESBL *E.coli*, after incubation period the zone of inhibitions for Ceftazidime (13, 14, 21), Ceftriaxone (20, 11, 14) and Cefotaxime (9, 9, 6) were according to CLSI recommendation (if bacterial strains showed the zone of inhibition ≤22 mm for Cefatazidime, zone of inhibition ≤25 mm for Ceftriaxone and zone of inhibition ≤27 mm for Cefotaxime then they are selected as ESBL producing *E.coli*. Results of double disc synergy test (DDST) were presented in Figure 1 and Table 1.

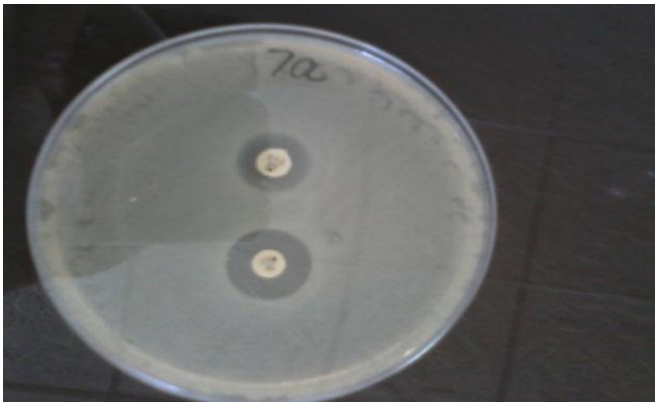

Figure 1: Double disc synergy test for ESBL *E.coli* confirmation.

Table 1: Zone of inhibition of antibiotics in Double Disc Synergy Test.

Zone of inhibition (mm)			
Bacterial Strain	**Cefotaxime (CTX-30)(mm)**	**Cefotaxime/Clavulanic Acid (CTC-30/10)(mm)**	**Difference (mm)**
ESBL *E.coli* strain I(PK-4)	9	15	06ESBL confirmed
ESBL *E.coli* strain II(5C)	9	14	05ESBL confirmed
ESBL *E.coli* strain III (7A)	6	16	10ESBL confirmed

The result showed that ESBL positive isolates of *E.coli* with a difference of zone diameter between Ceftazidime disc alone and Ceftazidime/Clavulanic acid disc ≥ 5mm. Results showed that detection of co-resistance among ESBL producing *E.coli* (Figure 2).

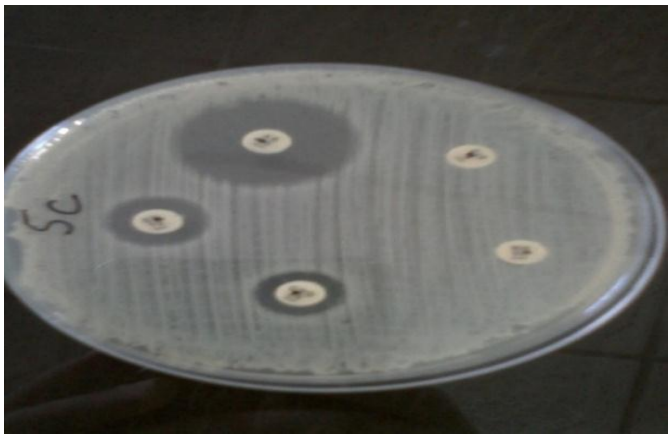

Figure 2: Antibacterial activity of different antibiotics against bacteria. The antibiotics with no zone of inhibition showed resistance pattern.

Table 2: Comparison of mean of zone of inhibition of different antibiotics against bacterial strains.

Antimicrobial discs	**Strains**			**Mean± SE**
	Strain 1	**Strain 2**	**Strain 3**	
AK	26	24	21	23.67±1.45 B
CN	10	13	6	9.67±2.03 C
CIP	7	6	6	6.33±0.33 D
NOR	7	6	6	6.33±0.33 D
NA	9	6	6	7.00±1.00 CD
IPM	30	26	29	28.33±1.20 A
AMP	10	6	6	7.33±1.33 CD
CAZ	22	21	21	21.33±0.33 B
F	26	30	25	27.00±1.53 A
CTX	9	9	6	8.00±1.00 CD
Mean	15.6±2.91A	14.70±3.03AB	13.20±3.02B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Figure 3 shows the result of PCR products. The ethanol extract of black pepper showed 29 mm inhibitory zone against *E.coli* while ethanolic extract of *thymus* showed 8 mm (Figure 4, Table 4). The ethanolic and aqueous extracts of both ginger and black pepper were statistically non-significant. However, treatment with thyme had somewhat different activity. Statistical results showed that both ethanolic and aqueous extracts are statistically non-significant, both of these extracts are similar (Table 5).

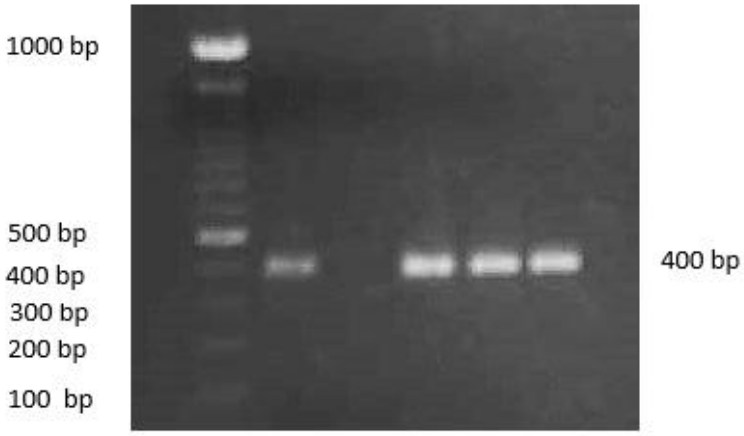

Figure 3: Plasmid profile of Ethidium bromide stained PCR product of the tested bacteria after gel electrophoresis. Lane 1: ladder (100bp), Lane 2: positive control (ATCC25922), Lane 3: negative control, Lane 4: test isolate I (bacterial strain I), Lane 5: test isolate II (bacterial isolate II), Lane 6: test isolate III (bacterial isolate III).

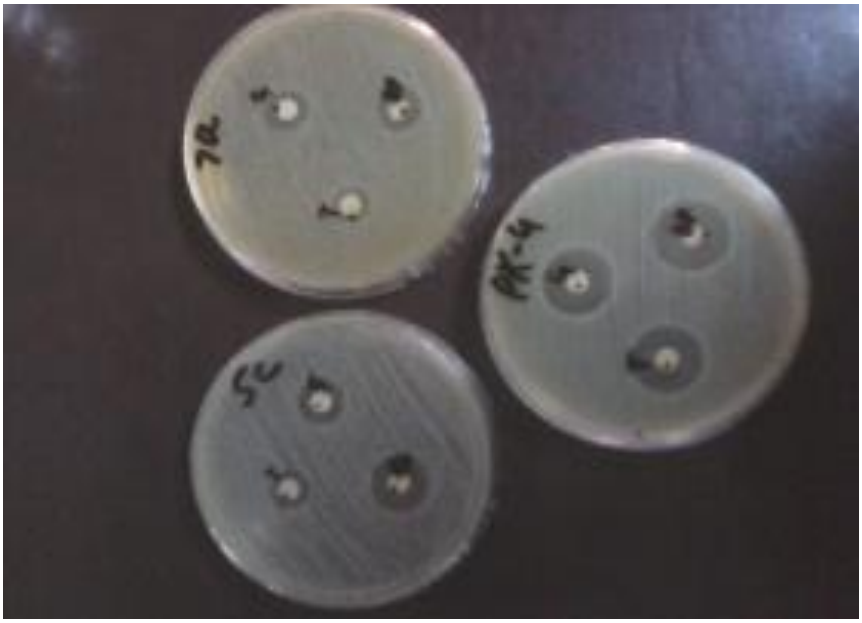

Figure 4: Antibacterial activity of plant extracts (Ginger, Black pepper, Thyme) against ESBL *E.coli* isolates.

Table 4: Susceptibility pattern of crude ethanolic and aqueous plant extracts against ESBL *E.coli* strains*.

Microbial strain	Susceptibility pattern of crude plant extracts					
	Ginger ethanol	Ginger aqueous	Black pepper ethanol	Black pepper aqueous	Thyme ethanol	Thyme aqueous
I (PK-4)	++	++	++	++	+	++
II (5C)	++	+	+++	++	+	+
III (7A)	++	+	+	+	+	+

*Diameter of zone of inhibition: 5 – 15 mm (+), 16 – 25 mm (++), 26 – 35 mm (+++).

Table 5: Phenotypic screening of ESBL *E.coli*, (mean ± SE).

Plant	Strains		
	Strain 1	Strain 2	Strain 3
Ginger	19.50 ± 0.50	16.00 ± 3.00	14.00 ± 2.00
Black pepper	19.50 ± 0.50	22.50 ± 6.50	12.00 ± 1.00
Thyme	14.50 ± 3.50	12.50 ± 1.50	9.00 ± 1.00
Total	17.83 ± 1.40A	17.00 ± 2.65A	11.67 ± 1.12B

The result also revealed that all three bacterial strains were treated with three selected plants i.e. ginger, black pepper and thyme. Strain 1 and strain 2 respond similarly to the applied treatments, whereas strain 3 response was slightly differ from them.

Table 6: Phenotypic screening of ESBL *E.coli* by plant extract (mean ± SE).

Extract	Plant		
	Ginger	Black pepper	Thyme
Ethanol	18.33 ± 1.20	19.67 ± 5.21	11.00 ± 1.73
Aqueous	14.67 ± 2.19	16.33 ± 2.03	13.00 ± 2.52
Total	16.50 ± 1.38A	18.00 ± 2.61A	12.00 ± 1.44B

The ethanolic and aqueous extracts of both ginger and black pepper was statistically non-significant i.e. they are same and the treatment with either of them produces almost similar results. While treatment with thyme had somewhat different activity. As mean of black pepper is greatest from the other two plants so black pepper showed greater antibacterial activity while thyme showed the least antibacterial activity amongst the selected plants that were used in this study.

Table 7: Phenotypic screening of ESBL *E.coli* treatment by strain (mean ± SE)*.

Strain	Extract	
	Ethanol	Aqueous
Strain 1	16.67 ± 2.85	19.00 ± 0.58
Strain 2	20.67 ± 4.41	13.33 ± 1.45
Strain 3	11.67 ± 2.33	11.67 ± 0.88
Total	16.33 ± 2.11A	14.67 ± 1.22A

*Means sharing similar letter in a column are statistically non-significant (P>0.05)

Results showed that both ethanolic and aqueous extracts were statistically non-significant and were similar.

Synergistic effects of plants and antibiotics were presented in Figure 5.

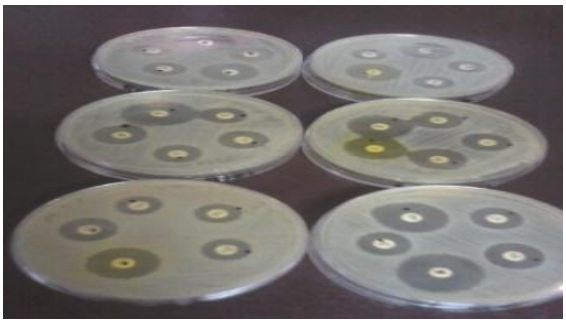

Figure 5: Zone of inhibition of antibiotics dipped in plant extracts for evaluation of the combined activity of antibiotics with plant extracts.

In Table 8, indicating resistance pattern of bacterial strain I towards antibiotics when treated with various antibiotics alone as well as when treated with antibiotics plus plant extracts. They shows susceptibility pattern. Thus the results indicated that plant extracts had remarkable antibacterial activity.

Table 8: Comparison of zone of inhibition of antibiotics alone with combined effect of antibiotics + plant extracts.

Antimicrobial discs (µg) (OXOID COMPANY)	ESBL *E.coli* strain I (PK-4)	ZONE OF INHIBITION (mm)					
		GINGER		BLACK PEPPER		THYME	
		Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Amikacin (AK-30)	26	27	27	27	27	26	25
Gentamicin (CN-10)	10	16	20	21	17	19	21
Ciprofloxacin (CIP-5)	7	28	27	31	28	27	26
Norfloxacin (NOR-10)	7	27	25	26	28	25	20
Nalidixic Acid (NA-30)	9	20	16	30	20	18	17
Imipenem (IPM-10)	30	35	38	33	32	34	36
Ampicillin (AMP-10)	10	18	19	31	20	25	20
Ceftazidime (CAZ-30)	13	24	24	25	20	29	24
Nitrofurantoin (F-300)	26	20	20	30	23	18	17
Cefotaxime (CTX-30)	9	30	30	32	30	28	29

Similar results were found (resistance pattern) with bacterial strain II and strain III towards antibiotics when treated with various antibiotics alone as well as when treated with antibiotics plus plant extracts (Table 9, 10 and 11).

Table 9: Comparison of antibiotics alone with combined effect of antibiotics + plant extracts.

Antimicrobial discs (µg) (OXOID COMPANY)	ESBL *E.coli* strain II (5C)	ZONE OF INHIBITION(mm)					
		GINGER		BLACK PEPPER		THYME	
		Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Amikacin (AK-30)	24	23	23	25	22	24	24
Gentamicin (CN-10)	13	17	16	25	18	17	17
Ciprofloxacin (CIP-5)	6	16	16	23	18	15	17
Norfloxacin (NOR-10)	6	18	16	21	18	15	16
Nalidixic Acid (NA-30)	6	18	15	22	18	21	16
Imipenem (IPM-10)	26	31	29	32	33	32	29
Ampicillin (AMP-10)	6	16	20	23	16	16	16
Ceftazidime (CAZ-30)	14	20	20	25	21	20	17
Nitrofurantoin (F-300)	30	21	23	24	21	22	22
Cefotaxime (CTX-30)	9	17	17	22	16	18	19

Table 10: Comparison of antibiotics alone with combined effect of antibiotics + plant extracts.

Antimicrobial discs (µg) (OXOID COMPANY)	ESBL *E.coli* strain III (7A)	ZONE OF INHIBITION (mm)					
		GINGER		BLACK PEPPER		THYME	
		Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Amikacin (AK-30)	21	23	22	26	20	22	21
Gentamicin (CN-10)	6	16	13	22	14	18	22
Ciprofloxacin (CIP-5)	6	16	14	24	15	16	18
Norfloxacin (NOR-10)	6	25	21	22	19	20	14
Nalidixic Acid (NA-30)	6	16	11	22	18	17	13
Imipenem (IPM-10)	29	30	32	35	34	32	31
Ampicillin (AMP-10)	6	16	14	21	18	17	13
Ceftazidime (CAZ-30)	11	20	21	24	22	23	22
Nitrofurantoin (F-300)	25	26	22	26	23	25	22
Cefotaxime (CTX-30)	6	18	15	24	17	20	19

Table 11: Comparison of means of the antibiotics alone as well as antibiotics plus plant extracts against all three bacterial strains.

		Mean						
		Gentamicin (CN-10)	Ciprofloxacin (CIP-5)	Norfloxacin (NOR-10)	Nalidixic Acid (NA-30)	Ampicillin (AMP-10)	Ceftazidime (CAZ-30)	Cefotaxime (CTX-30)
ESBL *E.coli*		9.67B	6.33B	6.33B	7.00C	7.33B	21.33A	8.00B
Ginger	Ethanol	16.33AB	20.00AB	23.33A	18.00AB	16.67AB	21.33A	21.67AB
	Aqueous	16.33AB	19.00AB	20.67A	14.00BC	17.67A	21.67A	20.67AB
Black Pepper	Ethanol	22.67A	26.00A	23.00A	24.67A	25.00A	24.67A	26.00A
	Aqueous	16.33AB	20.33AB	21.67A	18.67AB	18.00A	21.00A	21.00AB
Thyme	Ethanol	18.00A	19.33AB	20.00A	18.67AB	19.33A	24.00A	22.00AB
	Aqueous	20.00A	20.33AB	16.67AB	15.33B	16.33AB	21.00A	22.33AB

DISCUSSION

It has been reported that many strains of bacteria are resistant to antibiotics. The resistance of *E.coli* to antibiotic is increased probably because of development of CTX type extended spectrum β-lactamase (ESBL) genes. These ESBL producing bacteria are resistant to most of the 3rd generation cephalosporin. The occurrence of ESBL producing strains in nosocomial infection limit the antibiotics therapy.[18] The modification in micro-organisms due to genetic mutation leads toward the resistance in antibiotics.[19] The current study was performed to check the relationship of antibacterial activity of medicinal plants and target on the multi drug resistance among the species of *E.coli*. More than three antibiotics showed no response against bacterial strains. The study stated that these Gram negative bacteria are termed as multiple drug resistance (MDR). When these results were compared with the standard designed by

Clinical and Laboratory Standards Institute (CLSI), the drugs (mainly Norfloxacin, Ampicillin, Ciprofloaxacin, Gentamicin, Nalidixic acid and Ceftoaxime) exhibited resistance and ESBL strains were susceptible to Imepenem, Amikacin, and Nitrofurantoin by aid of Kirby-Bauer disk diffusion method. In nosocomial infections, Fluoroquinolone showed resistance against *E.coli*.[20] The medicinal plants are of great interest. The antibacterial activity of ginger, black pepper and thyme was assessed in this study and the results indicated that *E.coli* are sensitive to ethanolic and aqueous extracts of these plants with an inhibitory zone of 1 – 29 mm. Bacterial strains resist some antibiotics when given alone such as Gentamicin, Norfloxacin, Cefotaxime, Nalidixic acid, Ampicillin, Ciprofloxacin and Ceftazidime. But when these antibiotics were soaked in plant extracts their resistance pattern turns towards susceptibility. Thus treatment with antibiotics alone was significantly different from the treatment with combination of antibiotics plus plant extracts. The results of current study are in accordance to previous research studies.[21,22] There are many phytochemical properties of plants but mostly mode of action against a bacterium is unknown.[28] However, it is assumed, this may be due to aqueous extracted anionic components like Chlorides, Nitrate, Thiocyanate, Sulphates and many other naturally present compounds.[23] Different studies were made to check the difference in antibacterial activity by using different solvents. Hence, extraction of phytochemicals using ethanol gave best results compared to water and the reason was solubilization of many organic compounds.[24] Ginger extracts showed MIC of 9-14 mm. *P. aeruginosa* showed maximum inhibition at 14 mm and minimal inhibitory response of 9 mm against *E.coli*. A confirmatory statement that methanolic extract exhibited a good ZOI against *S. aureus* and *E.coli* was reported.[25] Moreover, results showed better susceptibility against medicinal plants leaving behind the antibiotics.[26] Ginger extracts exhibited moderate inhibition activity with the zone range of 9–14 mm. Maximum inhibition was detected against *P. aeruginosa* (14 mm) and minimum inhibition against *E.coli* (9 mm). Report also confirmed that ginger methanolic extract showed a major zone of inhibition against *E.coli and S. aureus*.[25] The result of this study showed similar result that plant extracts had much better antibacterial activities in contrast to antibiotics.[25] There is great worth in healing treatments by using plant extracts with recognized antimicrobial properties but some studies also reported numerous herbal medicine's contamination which may include microorganisms, pesticides and toxic heavy metals.[26] Therefore, sterilization is necessary especially for aqueous extracts to get rid of these impurities.[27,28]

The results of this study suggested that ginger, black pepper and thyme are strong bioactive and be beneficial in the disease therapy.

CONCLUSION

ESBL producing *Enterobacteriaceae* bacterial species are responsible for the progression of many infections throughout the world both in the hospital and community setups. The results of current research depict higher level of multi drug resistance in ESBL positive isolate of *E. coli*. The combined applications of antibiotics and plants extract have the potential to reduce the multidrug resistance bacteria (MDRs). The overall research results indicated that the ethanolic and aqueous extracts of the plants have good activity against bacteria and these extracts have the potential to increase antibacterial properties of antibiotics when used as cocktail. Still there are gaps to find out the active ingredients of these medicinal plants, their mode of action against bacteria. In future the combined preparations of antibiotics with plant extracts could be beneficial in reduction of drug resistance by the bacteria.

REFERENCES

1. Nanasombat S, Lohasupthawee P. Antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. KMITL Sci Techno J., 2005; 5: 527-538.
2. Ababutain IM. Antimicrobial activity of ethanolic extracts from some medicinal plant. Aus J. Basic Appl. Sci., 2011; 5(11): 678-683.
3. Bradford PA. Extended spectrum beta-lactamases in the 21st century: characterization, epidemiology and the detection of this important resistance threat. Clin Microbiol Rev., 2001; 14: 933-951.
4. Paterson DL, Bonomo RA. Extended-spectrum ß-lactamases: a clinical update. Clin Microbiol Rev., 2005; 18: 657-686.
5. Drawz SM, Bonomo RA. Review- Three decades of beta-lactamase inhibitors. Clin Microbiol Rev., 2010; 23(1): 160-201.
6. Drawz SM, Bonomo RA. Review- Three decades of beta-lactamase inhibitors. Clin Microbiol Rev., 2010; 23(1): 160-201.
7. Bush K. Review- Proliferation and significance of clinically relevant β- lactamases. Ann N Y Acad Sci., 2013; 1277: 84-90.
8. Seyyednejad SM, Maleki S, Damab NM, Motamedi H. Antibacterial activity of Prunusmahaleb and parsley (Petroselinum crispum) against some pathogen. Asain J Biol Sci., 2008; 1: 51-55.
9. Rottier WC, HS Ammerlaan, Bonten MJ. Effects of confounders and intermediates on the association of bacteraemia caused by extended spectrum beta-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. J Antimicrob Chemother, 2012; 67: 1311–1320.
10. Byarugaba DK. A view on antimicrobial resistance in developing countries and responsible risk factors. Int J Antimicrob Agents, 2004; 24(2): 105-10.

11. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science, 1994; 264: 375-382.
12. Wright GD. Bacterial resistance to antibiotics: Enzymatic degradation and modification. Adv Drug Deliv Rev., 2005; 57(10): 1451-1470.
13. Shinwari ZK. Medicinal plants research in Pakistan. J Med Pl Res., 2010; 4(3): 161-176.
14. Chawla R, Thakur P, Arora R. Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes. A dreadful lifestyle disorder of 21st century. J Diabetes Metab Disord, 2013; 12: 35 doi: 10.1186/2251-6581-12-35.
15. Van de Sande-Bruinsma N, H. Grundmann, D. Verloo. 2008. Antimicrobial drug use and resistance in Europe. Emerg Infect Dis., 2005; 14: 1722–30.
16. Souza EL, Stamford TLM, Lima EO, Trajano VN, Filho JB. Antimicrobial effectiveness of spices: an approach for use in food conservation systems. Braz Arch Biol Technol., 48: 549-558.
17. Cristani M, Arrigo MD, Mandalari G, Castelli F, Sarpietro MG, Micieli D, et al. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. J Agri Food Chem, 2007; 55: 6300-6308.
18. Aiyegoro OA, A.J. Afolayan, Okohx AI. Interactions of antibiotics and extracts of Helichrysumpedunculatum against bacteria implicated in wound infections. Folia Microbiol., 2010; 55: 176–180.
19. Ibrahim TA, Opawale BO, Oyinloye JMA. Antibacterial activity of herbal extracts against multi drug resistant strains of bacteria from clinical origin. Life Sci Leaflets, 2011; 15: 490–498.
20. Hamada TA, Mahmood AR, Ahmed IB. Antibiotic resistance in pathogenic bacteria isolated from utis in tikrit province. Tikrit Med J., 2008; 14(1): 203-210.
21. Odhav B, Juglal S, Govinden R. Spices oils for the control of co- occurring mycotoxins producing fungi. Euro Food Res Tech, 2002; 65: 683–687.
22. Masood N, Chaudhry A, Tariq P. Bactericidal activity of black pepper, bay leaf, anise seed and coriander against oral isolates. Pak J Pharmaceut Sci, 2006; 19(3): 214-218.
23. Al-aubadi IMK, Mousa MA, Abbas AJ. Chemical composition of thyme seeds Thymus vulgaris and its antimicrobial activity. Alanbar J Agricult Sci., 2011; 9(2): 294-305.
24. Darout I, Cristy A, Skaug N, Egeberg P. Identification and quantification of some potential antimicrobial anionic components in miswak extract. Ind J Phar., 2000; 32: 11-14.
25. Al-Daihan S, Al-Faham M, Al-shawi N, Almayman R, Brnawi A, zargar S, et al. Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. J Kin Saud Uni Sci., 2013; 25: 115–120.
26. Bele AA, Jadhav VM, Nikam SR, Kadam VJ. Antibacterial potential of herbal formulation. Res J Microbiol, 2009; 4: 164–167.
27. Talaly P, Talaly P. The importance of using scientific principles in the development of medicinal aspects of plants. Acad. Med, 2001; 76: 238–247.
28. Hashemi SR., Zulkifli I, Zunita Z, Somchit MN. The effect of selected sterilization methods on antibacterial activity of aqueous extract of herbal plants. J Biol Sci, 2008; 8: 1072–1076.
29. Lanciotti R, Gianotti A, Patrignani N, Belleti N, Guerzoni ME, Gardini F. Use of natural aroma compounds to improve shelf life of minimally processed fruits. Trends Food Sci Tech., 2004; 15: 201-208.