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# *Effect of Teucrium polium L Extract on Growth of E.coli in Liquid Media and Effect on Growth in Poisoned Food Technique*

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**ABSTRACT**

***Background:*** *Escherichia coli is a facultative anaerobic Gram-negative bacterium from the Enterobacteriaceae family that colonizes the gastrointestinal tract. Teucrium polium L is a medicinal plant growing in the western Mediterranean region. Different studies aim to determining the stages at which E. coli growthsis more sensitivity to certain concentrations of medicinal plant like Alcoholic and aqueous extract of Teucrium polium and their effective concentration based on the zone of inhibitions on plate that previously inoculate with Escherichia coli and the plant extracts and utilized to monitor growth of bacteria. in present study it was indicated that Teucrium polium L ethanolic and aqueous extracts was showed a significant outcome in inhibition of this bacteria.*

***Conclusion****: In different infection the role of E.coli is very important subjects, in present study it was indicated that Teucrium polium L ethanolic and aqueous extracts was showed a significant outcomes in inhibition of this bacteria, these data may be aid in discovering of new remedies from natural sources.*

***Keyword****: Escherichia coli, Teucrium polium, plant extracts, Poisoned Food Technique, Antibiotics.*

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## 1- INTRODUCTION

In 1885, Germany Austrian pediatricians Theodor Escherichia discovered the organisms in the fece of healthy individual. Called it Bacteria coli commune becausesits showed in the clon. Early classification of prokaryote place these in ahandful of genera according to their motility and shape (1 ). Bacteria coli was the types species of invalid Bacteria when it has been revealed that form types species was missed. Followed the revision of Bacteria, it has been re-classified as Bacilli by Migula in 1895s (2). *Enterobacteriaceae* has been original the soleefamily under an order 'Enterobacteria. The family contains alarge array of biochemical distinct species along to different ecological niche, which made biochemicals description difficults (3). The original classifications of species too the family and orders has been largely base on 16S r RNA genomes sequence analysis, which was know to having low discriminatory powers and the result of which change depend on algorithm and organisms information utilized. Despite the analysis still exhibits poly branching, indicated the presenc of distinct subgroup with in this family (4). In 2016, orders 'Enterobacteria' was renam to


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Enterobactera, and divide into seven new family, includs the emend Enterobacteriaceaeles families. The classification has been propose according to the constructions of various robust phylogenetic tree utilizing conserved genomes sequence (5).

In 2017, asubsequent study utilizing comparative phylogenomic analysis identify in the presence off sixs ubfamily levels clade with in the family Enterobacteriales, name *Escherichia* clades, *Klebsiella* clades, Enterobacter clades, Kosakonia clades (6). *E. coli* is one of the most common causes of diarrhea in children (7). After it was related to outbreaks of bloody diarrheal illness inin the state of Oregon and the state of Michiganin 1982, *Escherichia coli* was identified as a bacterium that causes food poisoning with serious and severe public health consequences. It was discovered by this scientist and named Bacterium coli to denote its normal presence in the intestines of healthy people. These bacteria were opportunistic pathogens that It has the ability to cause a variety of infections, including infant summer diarrhea, urinary tract infection (UTI, wound infection, meningitis, pneumonia, nosocomial infection and septicemia (8). This Special Issue “Pathogenic *Escherichia coli*: Infections and Therapies” consists of 12 articles, including original research on intestinal pathologies and extraintestinal infections of human and animal origins cautilized by *E. coli*, and a review on FimH antiadhesive molecules that can be utilized to treat urinary tract infections cautilized by uropathogenic *E. coli* (UPEC) (9).

### 1-1 Pathogenicity

One of the important reasons why colon bacteria were pathogenic is that they have multiple pathogenic factors, the most important of which is the fact that their cell wall contains lipopolysaccharides and secretion of two types of endotoxins: Heat Stable Toxin, non-antigenic origin and variable toxin. Heat Labile Toxin (Heat Labile Toxin) Antigens of Origin (10). It also produces the hemolytic enzyme, hemolysin, which plays a major role in its pathogenesis. These bacteria also have long velvets that enable them to attach to urothelial cells, as well as have peripheral flagella that enable them to attach to epithelial cells lining the intestines, and these bacteria were able to grow and multiply inside the host in addition to Unaffected by the immune system and resistance to the process of phagocytosis (11)

*E. coli* is one off the most significant model organism, and it is genetic and bio-chemistry was closely investigated. Some entero-bacteria were important pathogen, such as *Shigella* and *Salmonella*. because they produces endotoxin. Endotoxin resides in the cells wall and were releas when the cells dying and the cells wall disintegrate. Some member of the "Enterobacteriaceae" produce endotoxin that, when release in to the blood stream followed cell lyses, causes asystemic vasodilatory and inflammatory responses (12).

### 1-2 Escherichia coli resistance to antibiotics

Antibiotics were natural or manufactured secondary metabolites produced by microorganisms in the stationary phase and have the ability to inhibit other microorganisms without affecting the cells of the host body. Some of them have a limited spectrum, that is, they were specific to a specific type or group of microorganisms. Others have broad spectrum antibiotics, meaning that they work on various group of microorganism. Some of thems had a bactericidal effects, while other have bacteriostatic inhibitory effects. There were first discovere by Alexander Fleming at 1920s AD after discovering the drugs penicillin by chances (13). The antibiotic resistance of bacterium was

 

one of mostly important economic and health problem. In the worlds, which prompte researcher to investigates new antibiotic to overcomes the bacterial strains resistancy. Infections with resistance bacterium lead to a prolong treatment periods and increase risk of infections. There was several type of antibiotic resistances, includes multidrug resistant. This mean that bacterium were resistance to one of 3 antibiotic. Viability, and resistance of the type (XDR) means that the bacteria were resistant to two or all of the antibiotics taken, while resistance of the type (PDR) means that the bacteria were resistant to all antibiotics (14). Through many studies carried out by scientists in this regard, they found that the communication systems possessed by bacteria were responsible for the antibiotic resistance mechanism (15).

Oral antibiotics reduce the duration of AOM symptoms and consecutive MEE, but lead to adverse effects, like gastrointestinal symptoms and skin rash. Thus, current guidance recommends considering immediate antibiotics in these children .Immediate antibiotic treatment is recommended in those with AOM who were <6 months of age, immunocompromised or have craniofacial malformations, as well as those with severe illness due to AOM (16).

### 1-3 *Teucrium polium*

Treatments of different disease utilizing medicinal plant trace back to the ancient time. *T. polium*, the herbs that was utilized in the traditional medicines, in about 2000 year ago, was found principally in Mediterranean regions and various were from Iran particularly in semiarid part of the mountain and plain (17). This plants, belongs to Lamiaceae families and flower times was between August and June (Figure 1). The plant include three hndred species in the worlds, and was identify in tweelve species in republic of Iran. Among these, *Tecurium polium*, was utilized in traditional medicines. This plants was diuretic, antirheumatic, carminative and anti bacterial property (18).

**Figure (1) *Teucrium polium* plant (63)**

### 1-3-1 Antimicrobial Effect of *Tecurium polium*

*T. polium* aqueous extracts was antibacterial property, but antifungal effects has not confirm. The result demonstrate that *Tecurium polium* extracts significantly were anti-microbial activities invitro, particularly on strain of grampositive bacterium (19). Oil extracts from the *Tecurium polium* with minimum concentrations of 3 to 5 µl/ml, was the inhibitory effects on *B. cereus* bacteria, *E. faecalis* and *E. coli*. Raei etal. confirm the antibacterial activities of *Tecurium polium* is esential oil against *K. pneumoniae*. Motamedi etal (20) evaluate the antibacterial effectof *Tecurium polium* on *S. aureus* strain and they suggest that *Tecurium polium* was aneffective medicine plant for treatments of infections cautilize by *Staphylococcus aureus*. Sevindik etal. determine the anti-microbial activities and chemical compositions of *Tecurium polium* esential oil provide from the Anatolian. It was shown that the plant have an inhibition effects on resistant microorganism such as methicillinresistant *P. aeruginosa*, *Staphylococcus aureus*, , *Escherichia coli* Q157H7 and *Bacillus cereus* (21).

## 2-Materials and Methods

### 2-1 Sample collection

One hundred samples has been collected from Al-Zahraa teaching hospital in Iraq in a period of January 2021 to March 2022 according to Ilam university ethics committee. All sample were culture and identify by utilizing conventional microbiologic procedure. Sample positive for *Escherichia coli* which showes pink colony on MaConkey agar were choosed for this study. *E. coli* which reveal positive results in culture will be confirm by various biochemical test. Antimicrobial sensetivity ests and Phenotype detection of "ESBL" Anti microbial sensetivity test of *Escherichia coli* isolate was used on Mueler Hinton agars plate utilizing KirbyBauer agars method

### 2-2 Antibacterial Discs according to (22)

Nine antibiotic discs were utilized in this study as showed in table (1).

**Table (1): Antibiotic discs utilized in present study.**

| No. | Antibiotic | Code | Concentration µg/disk |
|---|---|---|---|
| 1 | Imipenem | IMP | 10 |
| 2 | Amikacin | AK | 30 |
| 3 | Gentamycin | GN | 30 |
| 4 | Ceftriaxone | CRO | 10 |
| 5 | Ceftazidime | CAZ | 20 |
| 6 | Nitrofurantoin | NIT | 30 |
| 7 | Cotrimoxazole | CO | 25 |
| 8 | Cefipime | CEF | 30 |
| 9 | Colistine | COL | 10 |

### 2-3 *Teucrium polium L* Isolates

*Teucrium polium L* plant was obtained from the State Board of Plant Protection - Ministry of Agriculture in Iraq, figure 2.

**Figure (2): *Teucrium polium L* whole plant.**

### 2-4 Identification and isolation of bacteria

All samples were cultured on Blood agar and MaCconkey agar plates. The resultant colonies in these media were subcultured to get pure culture. The recovered isolates were subjected to different morphological and biochemical tests for identification to the species level as described by Bergey's Manual for determinative Bacteriology (23).

### 2-4-1 Preparation of cultural media for bacterial growth

The culture media which were utilized in the current study been prepwere d according to the instructions of manufacturer, where it was identified on containers then sterile in autoclave at 121ºC, 15 min and 15 pound Per Squwere - Inch (24). Following the sterilization each agar and broth has been calculated cwere fully and then media poured on petri dish and incubated at 37ºC for 24 hour to ensure their sterility as ready for cultured and storaged in a refrigerator (25).

## Results

### 3-1 Sample collection

The bacterial routine procedures were utilized in sample collection and culturing of *E.coli*, species identification was relied on microscopic observation. Results of culture in current study were that from 100 collected samples 30 (30%) samples were *E.coli*, 50 (50%) samples were *staphylococcus aureus*, 10 (10%) samples were *Klebsiella pneumonia* and 10 (10%) samples were *Streptococcus*. Table (2). Figure 3: Percentage of bacterial species.

**Table 2 Distribution of bacterial species.**

| Bacterial isolates | Number (%) |
|---|---|
| E.coli | 30(30%) |
| Staphylococcus aureus | 50(50%) |
| Klebsiella pneumonia | 10 (10%) |
| Streptococcus | 10 (10%) |
| Total | 100 (100 %) |

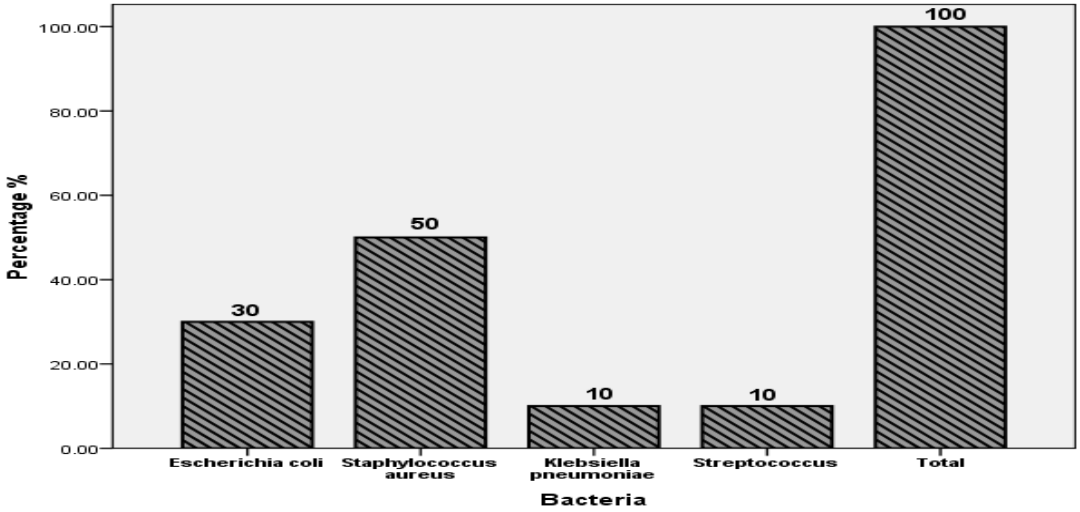


**Figure 3: Percentage of bacterial species.**

### 3-2 Colony morphology of *E.coli*

Bacterial isolate of *E.coli* were identified after 24 to 48 hours of aerobic incubation on MaCconkey and Blood agar plates at 35±2°C. This bacteria able to use lactose present in MaCconkey medium turned it to pink color. Colonies of *Escherichia coli* on MacConkey agar palte were pink to dark pink, dry and donut-shaped, surrounded by a dark pink were a of precipitated bile salts, figure (4 ). *Escherichia coli* was cultivated on blood agar. Cultivation 24 hours in an aerobic atmosphere, 37°C. Colonies were without hemolysis but many strains isolated from infections were beta-hemolytic.

 

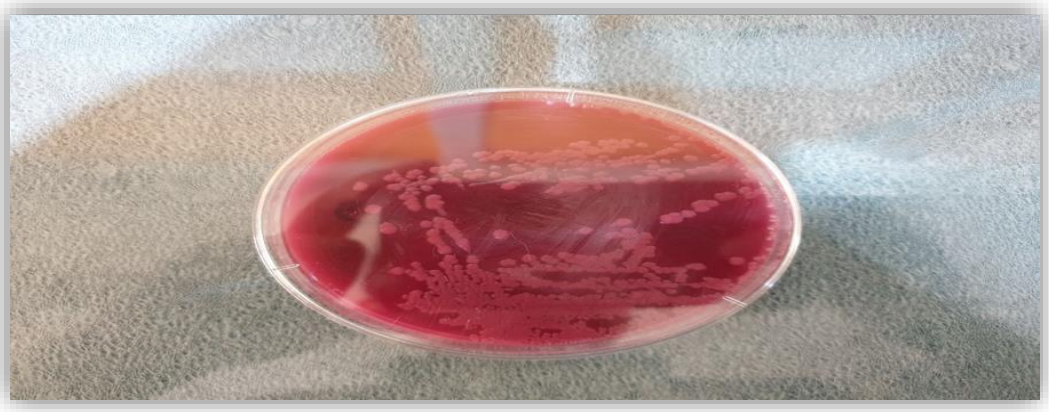

**Figure (4 ). E.coli growth on macConkey agar plate.**

### 3.3 Effect of *Teucrium polium L* extract on growth of *E.coli* in liquid media (broth)

Results of this experiment were observed in different concentration 2g/ml, 1g/ml, and 0.5g/ml. The results indicated that were was heavy growth in the 0.5 g/ml of both extracts, while there was no obvious growth in both 1 g/ml and 2 g/ml for both extracts,( figure 5).

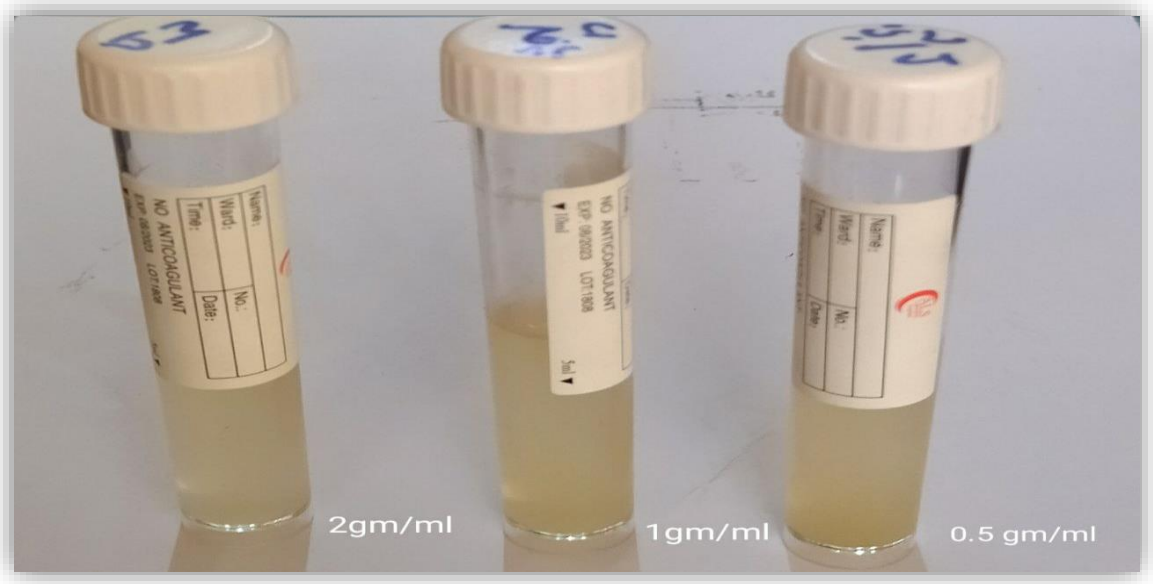


**Figure (5): Effect of *Teucrium polium L* ethanolic extract on the *E.coli* growth.**

### 3-4 Effect of *Teucrium polium L* extract on growth of *E.coli* in poisoned food technique

Outcomes of this procedure was showed in table (3) as the effect of ethanolic extract (23.00 ± 1.15 mm) at the concentration 0.5 g/ml with gentamycin (GEN, 30 µg), (30.00 ± 3.21 mm) at the concentration 1g/ml with Amikacin AK (30 µg) and (39.33 ± 2.02 mm) at the concentration 2 g/ml with imipenem (IMI, 10 µg), respectively in comparison to control, while for aqueous extract (20.33 ± 0.88 mm) at the concentration 0.5 g/ml with gentamycin (GEN, 30 µg), (25.66 ± 2.66 mm) at the concentration 1g/ml with Amikacin (AK, 30 µg) and (35.33 ± 2.02 mm) at the concentration 2 g/ml with imipenem (IMI, 10 µg). While antibiotics alone indicated the following results (18, 20 and 32mm) for GEN, AK and IMI respectively, these results revealed

 

no significant differences as p value= 0.319 for both extracts. The results showed in table 3 and figure 6 indicated that combination of antibiotics and extracts had a positive effect against *E.coli*, especially when combined with ethanolic extract.

**Table (3) Effect of *Teucrium polium L* ethanolic and aqueous extracts with antibiotic *E.coli* growth by poisoned food technique.**

| Plant extract | Concentration in (g/ml ) | Inhibition zone (mm) |
|---|---|---|
| | | E.coli |
| Ethanolic | 0.5 +30 GEN | 23.00 ± 1.15 $^{de}$ |
| | 1+30 AK | 30.00 ± 3.21 $^{bcd}$ |
| | 2+10 IMI | 39.33 ± 2.02 $^{a}$ |
| Aqueous | 0.5 +30 GEN | 20.33 ± 0.88 $^{e}$ |
| | 1+30 AK | 25.66 ± 2.66 $^{cde}$ |
| | 2+10 IMI | 35.33 ± 2.02 $^{ab}$ |
| GEN alone | | 18.00 ± 0.00 $^{e}$ |
| AK alone | | 20.00 ± 0.00 $^{e}$ |
| IMI alone | | 32.00 ± 0.00 $^{abc}$ |
| Control | | 0 |

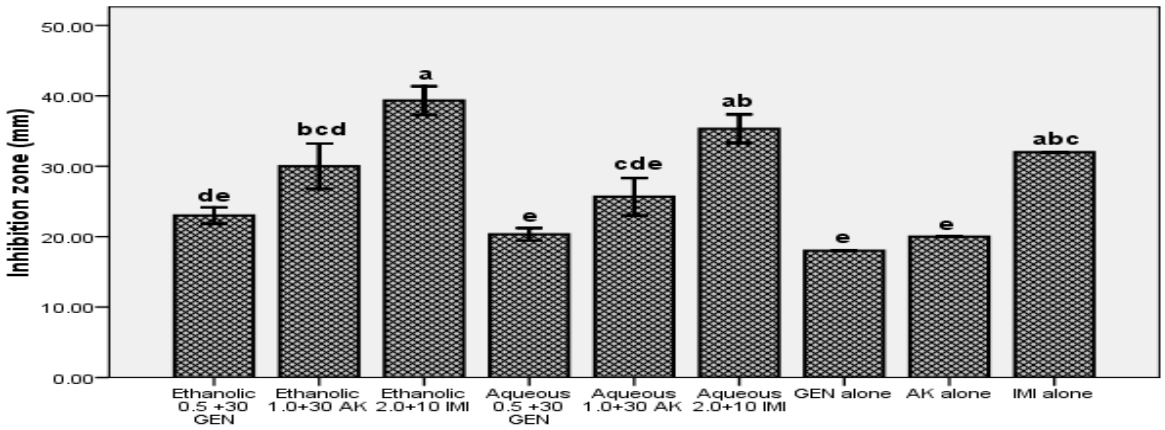


**Figure 6: Effect of Teucrium polium L. ethanolic and aqueous extracts with antibiotic on E.coli growth by poisoned food technique.**

### 3-4 Effect of other antibiotics on the *E.coli* growth by disc diffusion method

outcomes of this procedure was showed in table (4 ) as the effect of these antibiotics including Ceftriaxone, Ceftazidime, Nitrofurantoin, Cotrimoxazole, Cefipime and Colistine did not show significant inhibition zone when compwere d to GEN, AK and IMI.

**Table (4): The effects of other antibiotics in this study utilizing disc diffusion method.**

| Antibiotics | Concenrtation | Inhibition zone (mm) |
|---|---|---|
| **Ceftriaxone** | **10ug** | **12** |
| **Ceftazidime** | **20ug** | **14** |
| **Nitrofurantoin** | **30ug** | **10** |
| **Cotrimoxazole** | **25ug** | **9** |
| **Cefipime** | **30ug** | **13** |
| **Colistine** | **10ug** | **12** |

## 4. Discussion

### 4.1 Collection of Samples from patients.

Urinary tract infection (UTI) is the third most common infection of humans after respiratory and gastrointestinal infections (26). It has been estimated that about six million patients visit outpatient departments (OPD) and about 300,000 were treated in the wards every year for UTI worldwide (27). The causative agents of UTI were developing resistance against antibiotics and treatment costing the global economy. The prevalence of UTI has been reported in all age groups and in both sexes (28). *Staphylococcus aureus*, one of the most common causes of pyogenic infections is also a normal flora of the skin of human beings, it is a coagulase-positive, Gram-positive bacterium which is frequently implicated pathogen in bloodstream infections, skin and soft tissue infections, pneumonia, device-related infections and post-operative wound infections (29). The prevalence of S.aureus among clinical isolates is 19.96% (30).

### 4.2 Effect of *Teucrium polium L* extract on growth of *E.coli* in liquid media (broth).

Results of this experiment were observed in different concentration 2g/ml , 1g/ml , and 0.5 g/ml, the results indicated that were was heavy growth in the 0.5 g/ml of both extracts, while there was no obvious growth in both 1g/ml and 2g/ml for both extracts. The same results obtained by Shahba showed that *Teucriumpolium* extracts were effective in *E.coli* and *Pseudomonas* bacteria. In general, the MIC rate of aqueous extract in *Enterococcus* was 1.25 - 5 mg/mL. The MIC rate of ethanolic extract for *Enterococcus* was calculated as 10 mg/mL. The MIC of aqueous and ethyl acetate extracts for *Pseudomonas* bacteria were achieved at 5 and 20 mg/mL, respectively. The MBC contents of aqueous and ethyl acetate extracts of *Teucrium* for *Pseudomonas* bacteria was 10 mg/mL in aqueous and 20 mg/mL in ethyl

 

acetate extracts. The MBC content of extracts for *Enterococcus* bacteria were 10 mg/mL in aqueous extracts and 20 mg/mL in ethanolic extracts (31). The results of (32) study have represented the fine antibacterial activities of plant extracts that could be utilized as appropriate treatments for infections cautilized by *Escherichia coli* and *Pseudomonas aeruginosa*.

### 4.3 Effect of *Teucrium polium L* extract on growth of *E.coli* in poisoned food technique.

Outcomes of this procedure was showed that the effect of both extracts increased when combined with antibiotics. On basic of evidences obtain from study some general conclusion could be draw regards the effects of the plants materials on the activities of antibiotic utilized. Different plant sometime belongs to same families, have different effect on the activities of antibiotic (33). Different active compound were detect in aqueous mixed extracts of the three medicinal vegetations, includes phenol and flavonoid tannin, saponin and terpenes. Aqueous extrusions concerned the herbal agregate upon three medicinal vegetations, undergoes antibacterial effects against positivity but village gr-ve microorganisms. Consequences aid that aqueous extracts regards these 3 plants combination wellknown show the synergism effects as anti bacterial activities compare to previous study that examine each plants alone (34).

Gram positive microorganism may harbors transmissible genetic elements encods for antibiotic resistant such as plasmid encoding the penicilinase gene, namely in collects negative bacterium; conjugations was an esential mechanism regards drug transfering and effects ocur in to suprem genus (35). The trans peptidation responses was sensitive to pro hibition via βeta lactam. The penicillinsensitive reaction were catalyze by utilizing families of close related protein, penicillinbinding protein (PBP). Beta Lactam antibiotic out turns the lethal impacts regards bacterium via activation concerns, for this reason inhibiting cells wall syntheses (36). Several medicinal plant were utilized by human for the vast diversities in chemical compounds contents and anti microbial activities. Moreover, the appropriate using remain under tests in researchs. The effective concentrations "(0.15 to 0.4mg/ml)" were chosed, according to the zone of inhibitions on plate that were inoculate with *Escharichia coli* 157 and plants extract and utilized to monitor bacteria growth spectrophotometrically. All concentration of aerial part of *Tecurium polium* extracts and seed of *P.harmala* decrease an absorbance in first hour after the inoculation.

### 4.4 Effect of other antibiotics on E.coli growth by disc diffusion method

E. coli normally exist in intestine to be micro flora and play significant roles in absorption and digestion (37). On contrary Escherichia coli could be pathogens or zonotic, cautilize disease like hemorrhagic colitis and diarrhea (38). Antimicrobial were normally utilized in live stock in treating disease caused by Escherichia coli, like mastitis and diarrhea. Nevertheless, the utilizes of antimicrobial in treatments of live stock and as growth promoters were link to the developments of multi drug resistant Escherichia coli, threating to health (39). In deed, Escherichia coli isolat from live stock sample was reported to shown different resistance to variuos antimicrobial, includes tetracycline, this resistant phenomenons was also aneconomic burdens for farmer because off cost incurred in treatments failure and aprolonged periods of treatments in bacterial infection (40). In Emirates, the commonlly antibiotic utilized to treat animal include oxytetracycline and tetracycline (41).

The detections of antimicrobial resistant

 

was usually done phenotypicaly assesing the sensitivities to the antibiotic as recommend by CLSI and EUCAST. The molecular detections based on PCR was widely utilized in detection of the gene responsible for resistant against different antibiotic(42). Antimicrobials resistance bacterium were detected in avariety of domestic animal and environment. Study from MiddleEast highlighted the spreading of resistance organism in hospital and to leser extents in live stock and environments. Most of study Escherichia coli were correlated to human (43). In this study the effect of antibiotics including Ceftriaxone , Ceftazidime, Nitrofurantoin, Cotrimoxazole, Cefipime and Colistine did not show significant inhibition zone when compwere d to GN, AK and IMI.

## Conclusion

In different infection the role of *E.coli* is very important subjects, in present study it was indicated that *Teucrium polium L* ethanolic and aqueous extracts was showed a significant outcomes in inhibition of this bacteria, these data may be aid in discovering of new remedies from natural sources.

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