

Effect of *Teucrium polium L* extract on *E.coli* growth by poisoned food technique , disc diffusion and approach of the disc diffusion methods

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Abstract

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* growth is more sensitivity to certain concentration 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. The aim of this study was to evaluate the effect of plant extracts on *E.coli* growth.

Key words: *E.coli*, Herbal extracts, Poisoned food technique, Disc diffusion and approach of the disc diffusion methods.

1. Introduction

E. coli is facultative an-aerobic gram-negative bacteria from the Enterobacteriaceae family that colonizes the gastro-intestinal tracts of warm blood animal shortly after it births, and it's a lifelong colonizer of adult. *E. coli* persists as harmless commensals in mucous layers, interacts with hosts in a mutualistic manner [1]. Moreover, there was a certain strain with

pathogenic property that could cause diseases, and, in turn, non-pathogenic *E. coli* in intestine could eventually cause and contribute to diseases in compromised host. Pathogenic *Escherichia coli* strain were groups in to pathotype according to the virulence factor and clinical spectra. The extra-intestinal pathogenic *Escherichia coli* grouped to comprise the strain actualizing an infection outside intestinal tracts, mainly

infection in urinary tracts, in addition to wound infections, sepsis and meningitis [2, 3].

The strains were diverse genetically and resembled non-pathogenic *Escherichia coli* resides in intestinal tracts. Distinct from the commensals and other strain, diarrhoeagenic *Escherichia coli* (DEC) causes intestinal infection and harbored specific surface adhesin and another virulence factor. Moreover, they could be classified into the following well defined pathotype: enterohemorrhagic *Escherichia coli* (EAEC), entero-pathogenic *Escherichia coli* (EPEC), entero-toxigenic *Escherichia coli* (ETEC), enteroaggregative *Escherichia coli* (EHEC), entero-invasive *Escherichia coli* (EIEC), and neurotoxic *Escherichia coli* (NTEC) [4, 5]. In recent years, new pathotypes referred as adherent invasive *Escherichia coli* (AIEC) were recorded as being related to inflammatory bowel diseases, especially Crohn diseases. A gram-negative organism, *Escherichia coli* is resistant to many antibiotics, and highly risk *Escherichia coli* multi-resistant clones were emerging. Indeed, carbapenems resistant extend spectrum *beta lactamase* (ESBL) producing strain were considered as a critical priority by WHO as a bacterial pathogen for newly designed antibiotic [2, 6]. While antibiotic

treatment had profound effect on human microbiomes. Therefore, new strategies, like narrow-spectrum treatment, antiadhesive, phage therapies, or vaccinations, were applied [4, 7].

Due to the increasing in rates of infection with antibiotic resistance microorganism and side effect of some synthetic antibiotic. An interest in medicine plant as natural alternative to synthetic drug was considered. Plants are known to produce a variety of compounds to protect themselves against a variety of their own pathogens. Therefore, plants could serve to be antimicrobial substances [4]. "*Teucrium polium L.*" is a medicinal plant growing in western Mediterranean regions. *Teucrium polium* is a grass plant, durable, 10 to 30 cm in height and calloused white exteriors that ordinarily had dispersal in rocks and sand were of Europe zone [7]. *Teucrium polium* was utilized for different pathologic conditions like antidiabetic, antispasmodic, anti-inflammatory and antioxidant effects [6]. In view of the reported literature, the reputation of *Teucrium polium* plant has a considerable attention. The current study aim is to investigate the possibilities of the drugs in the treatments to urinary tract infection [5]. Moreover, the appropriate use remains under investigations by many researchers. different

study aimed to determining the stages at which *E. coli* growth was more sensitivity to certain concentration of medicinal plant like alcoholic or aqueous extract of *Teucrium polium* and their effective concentration based on the zone of inhibition of plate that has been inoculate with *Escherichia coli* and plants extract utilizing to monitors bacterial growth rates [8].

1.1 Antimicrobial effect of *Teucrium polium*

T. polium aqueous extracts have antibacterial property, but antifungal effects are not confirmed yet. The result demonstrates that *Teucrium polium* extracts significantly were anti-microbial activities invitro, particularly on strain of gram-positive bacterium [9]. Oil extracts from the *Teucrium polium* with minimum concentrations of 3 to 5 µl/mL, shows inhibitory effects on *B. cereus* bacteria, *E. faecalis* and *E. coli* [9,10]. Furthermore, the antibacterial activities of *Teucrium polium* is essential oil against *K. pneumoniae* was also reported by Motamedi and co-workers [10]. Motamedi reported the evaluating of the antibacterial effects of *Teucrium polium* on *S. aureus* strain as an effective medicine plant for treatment of infections that catalyzed by *Staphylococcus aureus*. S. M. Seyyednejad,

and H. Motamedi both determined the antimicrobial activities and chemical compositions of *Teucrium polium* essential oil provided from the Anatolian. Seyyednejad results showed that the plant has an inhibition effect on resistant microorganisms such as methicillin resistant *P. aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* Q157H7 and *Bacillus cereus* [10].

1.2 New insights about *Teucrium polium* roles in treatments of bacterial infection

Plants were and still are excellent sources for discovering or synthesizing new drug compounds. Recently, in a high part of the world, the extract of medicinal plants was utilized according to their antibacterial, anti-fungal, and anti-viral property [9-11]. In Iran, utilizing of natural resource goes back to origin, and many reports on these topics exist [11]. The anti-bacterial property of like plants was poorly understood and remains under debate [12]. However, an investigation of the anti-bacterial property of plants, particularly endemic plants, can had a benefit finding identified effective species control the growth off significant bacterial pathogens. An ethanolic *P. harmala* extract showed higher antibacterial activities against *S. aureus*. In addition, ethanolic *Teucrium*

polium extracts were highly antibacterials activities [13].

1.3 Aims of the current study

The current study involves four main aims that include the evaluation of the effect of *Teucrium polium* extract on *E.coli*. Estimating of the resulting *Teucrium polium* extract, and the common antibiotics alone. Moreover, study the interaction between *Teucrium polium* extract, and antibiotics on *E.coli* bacteria. Furthermore, studying the prevalence of *E.coli* in Wasit province, and finally testing of serial concentration of *Teucrium polium* extract against *E.coli* bacteria.

2. Materials and methods

One hundred samples were collected from Al-Zahraa Teaching Hospital that located in Wasit province, Iraq from January 2021 to March 2022 from patients with urinary tract infection. All samples were cultured and identified by utilizing conventional microbiological procedures. Positive samples for *Escherichia coli* which shows pink colony on MaConkey agar were chosen for this study. *E. coli* which reveals positive results in culture will be confirmed by various biochemical tests (sugar fermentation, catalase, nitrate reduction test

and indole). Antimicrobial sensitivity Test and the phenotypic detections of ESBLs. Antimicrobial sensitivity Test of *Escherichia coli* isolate has been performed utilizing Mueller Hinton agar plate u utilizing the Kirby Bauer methods, utilizing nine antibiotics including imipenem, amikacin, gentamycin, ceftriaxone, ceftazidime, nitrofurantoin, cotrimoxazole, cefepime and colistin.

3. Preparation of ethanolic, and aqueous extracts of *Teucrium polium*

To obtain *Teucrium polium* L extract, hot water was mixed with *Teucrium polium* L plant. The extraction was done as following, a 300 g leaf and stem powder of the plant obtained by finely grinding with a grain grinder and straw was macerated in 300 mL distilled water for aqueous extract, 300 mL ethanol (ethanolic extract) for 24 hours at room temperature and then the extracts were retrieved utilizing filter paper (0.5µm). Then, the filtrate is concentrated in rotavapor (Buchi Rotavapor type R-210) at 40 °C for 30 minutes to remove the solvent for the ethanolic extract, allowing a dry residue that is kept in a container in the shade at 4 °C until it is utilized.

4. Results and discussions

4.1 The effect of *Teucrium polium* L aqueous and ethanolic extracts on *E.coli* growth by poisoned food technique.

Aqueous and ethanolic extracts both are shown in table 1, as the effect of ethanolic extract (12.00 ± 1.15 mm) were determined at the concentration of 0.5 g/mL. Also, (13.66 ± 1.60 mm) at the concentration of 1 g/mL, and (16.66 ± 0.88 mm) at the concentration 2 g/mL, respectively in comparison to control. On the other hand, the aqueous extract showed (10.00 ± 0.57 mm) at the concentration of 0.5 g/mL, (11.33 ± 0.88 mm) at the concentration of 1 g/mL, and (16.00 ± 0.57 mm) at the concentration of 2 g/mL. Moreover, both aqueous and ethanolic extracts effects are also demonstrated in (figure 1). These results revealed no significant differences when the p value is equal to 0.221 between both extracts. However, there are significant differences when the p value is lower than 0.05 between different concentrations in both extracts.

Table 1: The effect of *T. polium* aqueous and ethanolic extracts against *E.coli* growth.

Planet extract concentration in (g / mL)		Inhibition zone (mm) <i>E. coli</i>
Ethanolic	0.5	12.00 ± 1.15^b
	1	13.66 ± 1.60^b
	2	16.66 ± 0.88^a
Aqueous	0.5	10.00 ± 0.57^b
	1	11.33 ± 0.88^b
	2	16.00 ± 0.57^a
Control	0	

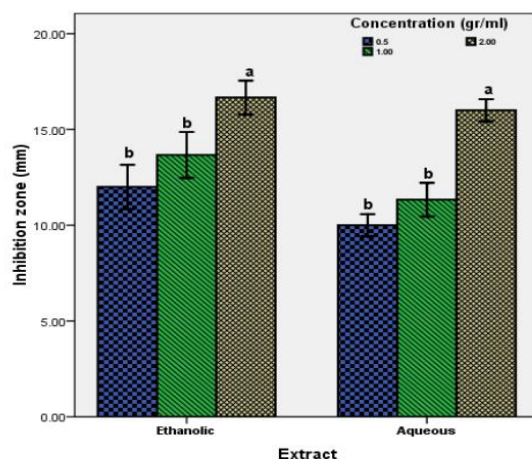


Figure 1: The effect of different extracts and concentration of *Teucrium polium* L.

4.2 The effect of *Teucrium polium* L extracts on growth of *E.coli* by disc diffusion method

Disc diffusion method outcomes are shown in (table 2) as the effect of the ethanolic extract are (9.33 ± 0.33 mm) at the concentration of 0.5 g/mL, (11.66 ± 1.20 mm) at the concentration of 1 g/mL, and (16.00 ± 0.57 mm) at the concentration of 2 g/mL in

comparison to control. While the aqueous extract is (8.00 ± 0.57 mm) at the concentration of 0.5 g/mL, (10.00 ± 0.57 mm) at the concentration of 1g/mL, and (13.33 ± 0.88 mm) at the concentration of 2 g/mL. These results revealed no significant differences as the p value is equal to 0.182 between both extracts. However, the significant differences between different concentrations in both extracts appear when p value is lower than 0.05 as shown in (table 2), and (figure 2).

Table 2: The effect of *T. polium* aqueous and ethanolic extracts against the *E.coli* growth.

Planet extract concentration in (g / mL)		Inhibition zone (mm) <i>E. coli</i>
Ethanolic	0.5	9.33 ± 0.33^b
	1	11.66 ± 1.20^b
	2	16.00 ± 0.57^a
Aqueous	0.5	8.00 ± 0.57^b
	1	10.00 ± 0.587^b
	2	13.33 ± 0.88^a
Control	0	

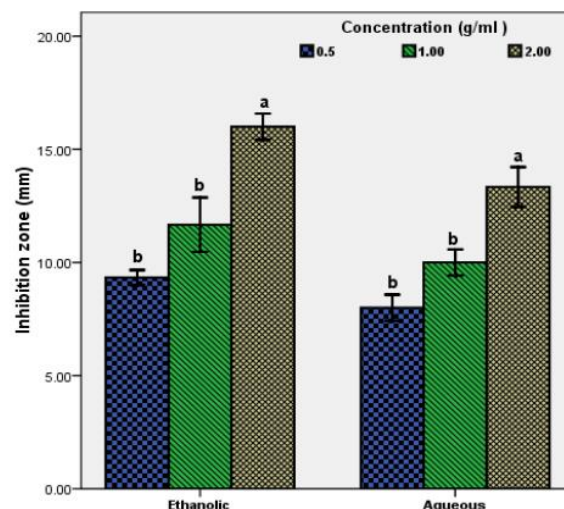


Figure 2: The effect of different concentration of *Teucrium polium* L. extract on growth of *E.coli* by disc diffusion method.

4.3 The effect of *Teucrium polium* L with antibiotic on *E.coli* growth by the approach of the disc diffusion methods.

The outcomes of this procedure are showed in table 3 as the effect of ethanolic extract is (21.33 ± 0.88 mm) at the concentration of 0.5 g/mL with gentamycin (GEN, 30 µg), (29.33 ± 2.96 mm) at the concentration of 1 g/mL with Amikacin (AK, 30 µg), and (39.00 ± 1.73 mm) at the concentration of 2 g/mL with imipenem (IMI, 10 µg), respectively in comparison with control. Furthermore, the aqueous extract showed (19.33 ± 0.88 mm) at the concentration of 0.5 g/mL with gentamycin (GEN, 30 µg), (25.66 ± 2.18 mm) at the concentration of 1g/mL with

Amikacin (AK, 30 µg), and (35.33 ± 1.45 mm) at the concentration of 2 g/mL with imipenem (IMI, 10 µg). Unalised antibiotics indicated the following results of 18, 20, and 32 mm for GEN, AK and IMI respectively. These results revealed no significant differences as the p value is equal to 0.412 for both extracts. Finally, table 3, and figure 3 both indicated that the combination of antibiotics and extracts had a positive effect against *E.coli*, especially when combined with ethanolic extract.

Table 3: The effect of *Teucrium polium L* ethanolic and aqueous extracts with antibiotic on *E.coli* growth by disc diffusion method.

Planet extract concentration in (g / mL)		Inhibition zone (mm) <i>E. coli</i>
Ethanolic	0.5 + 30 GEN	21.33 ± 0.88 ^{de}
	1 + 30 AK	29.33 ± 2.96 ^{bc}
	2 + 10 IMI	39.00 ± 1.73 ^a
Aqueous	0.5 + 30 GEN	19.33 ± 0.88 ^{de}
	1 + 30 AK	25.66 ± 2.18 ^{cd}
	2 + 10 IMI	35.33 ± 1.45 ^{ab}
GEN		18.00 ± 0.00 ^e
AK		20.00 ± 0.00 ^{de}
IMI		32.00 ± 0.00 ^{abc}
Control		0

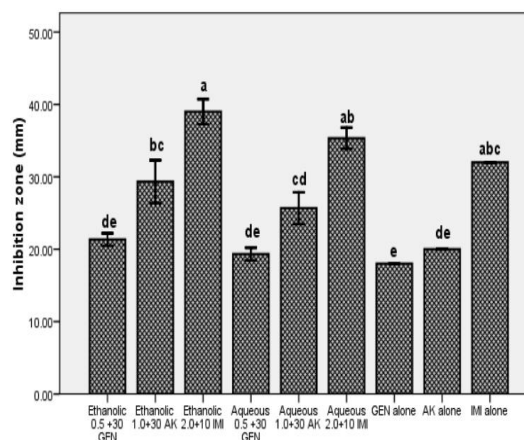


Figure 3: The effect of *Teucrium polium L*. ethanolic, and aqueous extracts with antibiotic on *E.coli* growth by disc diffusion method.

5 Discussion

The current study results on *E.coli* show a high agreement in comparison with Khalil A. et al. published results [14]. Khalil A. results distribution and susceptibility of UTI actualizing pathogens change according to time and place. Amongst 300 suspected UTI patients were referred by physicians from July to December 2012. The midstream urine samples were analyzed utilizing a semiquantitative culture method and uropathogens. Out of the 300 investigated samples, *E. coli* was isolated in 143 (47.7 %) samples, while 28 (9.4 %) samples showed no growth, 26 (8.7 %) samples showed mixed growth, 32 (10.7 %) samples were *Klebsiella pneumoniae*, 41 (13.7 %) were *Enterococci*

spp. and 30 (10 %) were coagulase negative Staphylococci. In gender wise distribution more females (60 %) were referred, and infestation of *E. coli* was also higher in females (66.5 %). The most infective age group was from 21 to 30 years 20 (21.1 %). In addition, in comparison to other causative agents. Similar results have been obtained by Sharma A. R. and Ahmed K. [15, 16]. However, the current outcomes did not agree with results that published by Odongo et al. [17]. Odongo reported that in 100 patients studied *E. coli* was the most dominant with a prevalence of 10 %. . Kabugo et al. published that with time, tremendous improvements in management of UTIs and community hygiene could have contributed to reduction of the prevalence of *E. coli* organisms [18].

5.1 The effect of *Teucrium polium* L aqueous and ethanolic extracts on *E.coli* growth by poisoned food technique.

Medical treatments using plant extracts have great attention for example the use of *Teucrium polium* L, because of the extract resistance and its effect on microorganism of pathogen that shown in facing of antibiotic. The antibacterials compound from the plant were used

extensively as medical resources. As a result of this spread of infections, exploration to more compounds will be interesting and helpful. Antibacterial compounds with herbal source had wide ranges of therapeutic utilize [19]. These compounds were not only efficient compounds in treatments of infectious disease, but concurrently diminishing existing side effect through the antibacterial compound. *E. coli* is a pathogens bacterium transmitted by infected foods. A worldwide epidemic of the disease was reported [20]. The third hospital infections factor after *E. coli* and *S. aureus* is *P. aeruginosa*. That showed sensitivities to different antibiotics, such as ciprofloxacin and piperacillin [21].

Results of this procedure revealed increased activity with increase concentrations in both extracts. The current data shows a high agreement with Jahani S. et al. results [22]. Extracts of *Teucrium polium* against *E.coli* were more effect in comparison with other extracts. The investigations of antimicrobial activity of plants extract shows that extremities of side effect on chemical drug efforts in finding alternative. Therefore, scientists have recently tried to find a new herbal drug without side effects. Moreover, recent studies showed that the antibacterial effect a few

types of *Teucrium polium* extract before gr+ve bacterium such as *B. cereus*, *B. subtilis*, *M. luteus*, and *S. aureus*. Gr-ve ones, such as *Escherichia coli*, *K. pneumonia*, *P. mirabilis* and *S. enteridis* also were studied. Based on Durmaz H, and Darabpour E. results [23-24] methanol extract shows a high antibacterial effect.

5.2 Effect of *Teucrium polium* L extracts on growth of *E.coli* by disc diffusion method

Teucrium belongs to the *Lamiaceae* family, with the sub family *Ajugoideae*. In flora off Europ this genus was divide in to 6 sections with forty-nine species. The sections *Stachybotrys* was represented by species *T. arduini* [25]. *T. polium* has a wide variety of secondary metabolites like tannin, alkaloid and flavonoid, which was found invitro to having anti-microbial property [26]. The outcomes of this procedure were showed ethanolic extract was more effective than aqueous extract at all concentrations. The current study results do not agree with Akin M. et al. results [27], because of that the ethanolic extracts of *T. polium* showed that *Bacillus anthracis* is the most sensitive species, while *Escherichia coli* and *Proteus mirabilis* were more resistant. In another study aimed at studying the effect of the

essential oils of *T. polium* plants obtain by hydro distillation on different bacterium and yeast, it was recorded that 4 μ l and 2 μ l application of the essential oils have no effects on *Escherichia coli* [28]. In 2012 Alizadeh-Behbahani B et al. reported that ethanol extracts in comparison to aqueous extracts are more effective and shows a great deterrent. This is perhaps due to extraction effectivity by ethanolic solution [29]. Anti-microbial activities of *T. polium* extract was attributed to various bioactive secondary metabolite. Other researchers utilized disc diffusion methods [30].

5.3 Effect of *Teucrium polium* L with antibiotic on *E.coli* growth by the approach of the disc diffusion methods

Urinary tract infections are the most common infections after respiratory tracts infection worldwide. Researchers showed a high interest in natural products with bactericidal activities when combined with antibiotics. The use of plant extract as a medicine against a variety of diseases in developed countries. This method depends on traditional medicine practitioner and their collection regarding medicinal vegetations in conform with treatments [31]. Herbals

execute applying significant positions in conserved biodiversity. Plants were genuinely familiar to bucolic humans being anybody according to the shortage. Indeed, medicinal plants lead to the very necessary health positions and then symbolizing against sources concerning profit for different family within the cities and countryside [32]. This procedure outcomes were showed that the effect of ethanolic extract at the concentration 0.5 g/mL with gentamycin GN (30 ug), Amikacin AK (30 ug) and imipenem IMI were less than concentration 2 g/mL with (10 ug) for the same antibiotics. The current study results have a high agreement with Darwish R. M. et al. results [33]. Darwish published results involved nineteen Jordanian plant extracts including *Teucrium polium* that were combine with antibiotic, from different class, and inhibitory effects of the combination was estimate. Methanolic extract of plant material enhances the inhibitory effect of neomycin and chloramphenicol against standard strains and to lower extents the resistance strains of *Escherichia coli*. While combination of amoxicillin with another plants extract utilized showed variable effects between standards and resistant strain. The current study probable suggests possibilities of concurrent utilize of the antibiotic and plant

extract in treating infection caused by *Escherichia coli* and at least the concomitants administrations might not impair the antimicrobial activities of such antibiotic [34, 35]. This strategy was named herbal shotguns and referred to the utilize of herbal and drug in multi target approaches, due to the facts that multi-extract combination affected not only one but also several target [36].

6 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 outcome in inhibition of this bacteria, these data may be aid in discovering of new remedies from natural sources.

7 References

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