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تقييم تأثير مستخلص أوراق الشاي الأخضر على مقاومة الاشريكية القولونية للمضادات الحيوية

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المستخلص:

المقدمة: تمثل مقاومة المضادات الحيوية لدى بكتيريا الإشريكية القولونية (*Escherichia coli*) تحدياً متزايداً للصحة العامة، إذ تحدّ من الخيارات العلاجية المتاحة لعلاج العديد من الالتهابات، ولا سيما التهابات المسالك البولية (UTIs) وقد حظيت المنتجات النباتية الطبيعية، مثل الشاي الأخضر (*Camellia sinensis*)، باهتمام متزايد نظراً لقدرتها المحتملة على التأثير في حساسية البكتيريا للمضادات الحيوية، ويُعزى ذلك بشكل رئيسي إلى احتوائها على مركبات بوليفينولية نشطة بيولوجيا **الهدف:** هدفت هذه الدراسة إلى تقييم التأثير المحتمل لمستخلص الإيثانول من أوراق الشاي الأخضر على نمط حساسية المضادات الحيوية لعزلات بكتيريا *E. coli* تحت الظروف المختبرية. **المواد وطرائق العمل:** تم استخلاص أوراق الشاي الأخضر باستخدام الإيثانول ثم أُعيد إذابة المستخلص في محلول ثنائي ميثيل سلفوكسيد (DMSO) بتركيز ١%. جرى اختبار خمسة تراكيز من مستخلص أوراق الشاي الأخضر بالإيثانول هي: ٠,٦٠ و٠,٥٠ و٠,٣٠ و٠,١٦ و٠,٠٨ غرام/مل. تم التعرف على عزلات *E.coli* باستخدام الاختبارات الكيميائية الحيوية القياسية، ثم تم تحليلها باستخدام جهاز **VITEK 2 Compact** لتحديد خصائصها الحيوية، إضافة إلى تقييم حساسيتها لمجموعة من المضادات الحيوية المختلفة. **النتائج:** أظهرت النتائج أن تعريض عزلات *E. coli* لمستخلص أوراق الشاي الأخضر بالإيثانول بتركيزين ٠,١٦ غرام/مل و٠,٠٨ غرام/مل أدى إلى حدوث تغيّرات ملحوظة في نمط حساسيتها للمضادات الحيوية. وقد تمثلت هذه التغيرات في زيادة المقاومة لعدة فئات من المضادات الحيوية، مع تحول بعض العزلات من نمط الحساسية العام إلى نمط مقاومة موسعة للأدوية (**Extensively Drug-Resistant, XDR**) تحت الظروف التجريبية. **الاستنتاج:** تشير نتائج هذه الدراسة إلى أن مستخلص الإيثانول لأوراق الشاي الأخضر قد يؤثر في نمط مقاومة المضادات الحيوية لدى بكتيريا *E. coli* في المختبر. وتُبرز هذه النتائج احتمال وجود تفاعل بين المركبات النباتية وآليات مقاومة البكتيريا للمضادات الحيوية، مما يستدعي إجراء المزيد من الدراسات لفهم الآليات الكامنة وراء هذه الظاهرة وتقييم أهميتها المحتملة من الناحية السريرية.

الكلمات المفتاحية: الشاي الأخضر، نمط مقاومة المضادات الحيوية، الإشريكية القولونية، ونظام VITEK 2 المدمج.

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Evaluation of the Effect of green Tea leaf Extract on the Antibiotic Resistance of Escherichia.coli

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Abstract:

Background: Antibiotic resistance in *Escherichia coli* is a major concern for public health, as it reduces options to treat infections such at urinary tract infection (UTI). Green tea (*Camellia sinensis*) and other natural plant products have been of interest for their ability to modify antibiotic susceptibility of bacteria, a capacity largely attributed to bioactive polyphenols. **Objective:** The purpose of this work was to investigate in vitro the potential effect of green tea leaf ethanol extract on the antibiotic susceptibility of *E. coli* isolates. **Materials and Methods**: Green tea leaves was extracted using ethanol and re-dissolved 1% DMSO. Five concentrations of green tea leaves ethanol extract 0.60, 0.50, 0.30, 0.16 and 0.08 g/mL were studied. *E. coli* isolates were detected by biochemical procedures and further tested using a VITEK 2 compact analyzer according to their biological profile; susceptibility to different antibiotic types was also established**. Results:** Exposure to green tea leaf ethanol extract at 0.16g/ml and 0.08 g/mL concentrations was associated with notable changes in the antibiotic susceptibility profile of *E. coli*. The observed alterations included a shift from a generally susceptible profile to an extensively drug-resistant (XDR) phenotype under the experimental conditions. **Conclusion:** Findings suggest that green tea leaf ethanol extract may modulate *E. coli* antibiotic resistance profile in vitro, indicating potential relationship between plant compounds and microbial antibacterial resistance mechanisms necessitating additional exploration

Keywords: Green tea, Antibiotic resistance pattern, *Escherichia.coli* and VITEK 2 compact system

Introduction

Antibiotic resistance has become one of the most serious public health problems worldwide due to its potential to reduce the efficacy of antimicrobial treatment and elevate morbidity, mortality, and healthcare costs (Estany-Gestal et al., 2024; Patra, M. 2025). *Escherichia.coli* is one of the leading source of both community and hospital-acquired infections, especially Urinary tract infection, sepsis and gastro-intestinal diseases (Govindarajan et al., 2024; Dziuba et al., 2025). The spread of multidrug (MDR) and extensively drug resistant (XDR) *E. coli* strains has limited the number of available therapeutic agents for treatment, therefore the lines of research aiming to investigate alternatives or adjuvant strategies to these health problems, represent a major breakthrough (Dadgostar, 2023).

E.coli has a high capacity for genetic adaptation, facilitating its spread and the acquisition of resistance determinants, such as point mutations, horizontal gene transfer (HGT), β-lactamase production, efflux pump overexpression and changes in membrane permeability (Cui X, et al., 2021). These mechanisms are involved in resistance to several class of clinically relevant antibiotics including β-lactams, fluoroquinolones, aminoglycosides, carbapenems, polymyxins and antifolate agents (Uddin et al., 2021).

Followed by an increase of antibiotic resistance, a growing interest has been aroused in natural plant derived compounds for their versatile biological activities. Green Tea (*Camellia sinensis*) Leaf is consumed worldwide as a popular beverage that contains bioactive constituents, especially polyphenols (e.g., catechins), vitamins and amino acids (Tallei et al., 2021; Zhao et al., 2022). Antimicrobial, antioxidant, and anti-inflammatory activities of green tea extracts were reported in the past that might explain the effect on bacterial physiology (Alghamdi, 2023).

A few studies have reported that extracts of plant can develop synergy or antagonism in antibiotic activity through impinging on bacterial stress responses (Vaou et al., 2022), membrane permeability, efflux pump systems or expression of genes associated with resistance mechanisms Nevertheless, very little experimental evidence exists about whether tea leaf extract exposure would modulate the antibiotic susceptibility against *E. coli* and influence the minimum inhibitory concentration ,including what direction would be synergy or antagonism and the extent to which one such modulation might be realized (Atta et al. (2023). This uncertainty is an important deficit in the current knowledge, particularly as a large proportion of the population consume green tea regularly and many have become interested in its medicinal uses (Wang et al., 2023).

Thus, the current study explores the effect of ethanolic green tea (*Camellia sinensis*) leaf extract on antibiotic resistance pattern of clinical isolates of *E.coli* under in vitro condition. In particular, this study attempts to unveil the effect of different concentrations of extract on the susceptibility patterns of *E. coli* isolates against antibiotics from various classes. Antibiotic susceptibility testing was carried out using the VITEK 2 Compact system. Green tea extract, rich in bioactive constituents, was hypothesized to affect pathogenic resistance through direct or indirect effects on physiological mechanisms involved in antimicrobial resistance.

Materials and Methods

Sample Collection

Patients who are clinically diagnosed with UTIs (urinary tract infections) were attending those samples at Al-Kindi Teaching Hospital, Shaikh Zayed Hospital, and Imam Ali Hospital in Baghdad city from whom urine samples were taken. Urine samples from patients with UTIs were collected under aseptic conditions using clean containers. To reduce contamination sterile, gloves were used. All samples were immediately taken to the microbiology laboratory for further treatment. Samples were not collected for epidemiological evaluation of UTI but solely to acquire *E. coli* isolates for use in vitro susceptibility testing.

Preparation of Green Tea Leaves

Dry, green tea leaves (*Camellia sinensis*) available on commercial markets and prepared for human consumption (food-grade product) were obtained from the local market in Baghdad - Iraq. Using a clean commercial blender, leaves were grinded into powder and stored in sterile airtight containers at room temperature pending extraction

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Preparation of Ethanol Green Tea Extract

Alcoholic extraction was used at 96% ethanol, being is efficient for extracting polyphenols from plant materials. Briefly, 500 g of powdered green tea leaves were soaked in 1000 mL of 96% ethanol in a sterilized glass container and subjected to constant shaking at room temperature for 48 h. The mixture was first filtered through sterile gauze to remove coarse particles and then further filtered using sterile Whatman filter paper No. 1. The obtained filtrate was evaporated to dryness at a controlled low temperature using a drying oven in order to completely remove residual ethanol without damaging heat-sensitive bioactive compounds (Plaskova et al., 2023; Hu et al., 2022). Complete evaporation of ethanol was confirmed before reconstitution to avoid any possible residual alcohol effect on bacterial viability or antibiotic activity. The dried extract was stored in tightly sealed screw-cap bottles at 4°C until further use (Onetto et al., 2024). The prepared extract was used without pH adjustment in order to preserve the natural chemical composition of the phytochemical constituents, and no additional chemical modification was introduced prior to antimicrobial testing

Preparation of Stock and Test Concentrations

The dried green tea extract was dissolved in 1% (v/v) dimethyl sulfoxide (DMSO) to generate a stock solution of 3.7 g/15 ml DMSO was chosen as a solvent as it is biologically inert at concentrations ≤1% and does not possess any antibacterial effect nor interferes with the antimicrobial susceptibility test (Gupta et al., 2022; Summer et al., 2022).

Five concentrations 0.60, 0.50, 0.30, 0.16 and 0.08 g/mL were prepared from the stock solution in an aseptic condition as working concentrations (Horablaga et al., 2023).

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E. coli Isolation and Identification

Urine cultures were inoculated on blood agar, MacConkey agar and eosin methylene blue (EMB) agar and incubated aerobically at 37°C for 18–24 h. Isolates presumptively identified as *E. coli* showed the following features: colony morphology, lactose fermentation in MacConkey agar, metallic green sheen on EMB agar and standard biochemical tests such as Gram staining, oxidase, catalase, indole production; utilization of citrate; and urea test. These tests were performed to achieve reproducible and accurate presumptive identification (Cappuccino, J., & Welsh, C. (2020).

E.coli Identification of VITEK 2 Compact

E. coli isolates were finally confirmed by VITEK 2 compact system (bioMérieux, France). Bacterial suspensions were prepared in sterile 0.45–0.50% (w/v) NaCl solution and standardized to a 0.5 McFarland with the VITEK 2 DensiCHEK Plus. The adjusted suspensions were inoculated into VITEK 2 ID-GN cards and incubated as per the manufacturer's recommendations.

Antibiotic Susceptibility Testing

Minimum inhibitory concentrations (MICs) for the *E. coli* isolates before and after being exposed to green tea leaf ethanol extract were detected by VITEK 2 compact system for susceptibility testing of antibiotics. The results of susceptibility were read following EUCAST guidelines.

Statistical analysis

Statistical analysis was not preformed due to the limited number of isolates.

Results

Isolation and Identification of *E.coli*

From the urine samples of patients diagnosed clinically as UTIs, one isolate was proved to be *E.coli*. Isolate identification was performed by cultural features on selective and differential media, biochemistry, and VITEK 2 compact system. The isolate had *E.coli* characters, such as fermenting lactose on MacConkey agar, giving metallic green sheen on EMB agar, Gram-negative reaction and was catalase and indole test-positive. As shown in Table (3.1). Bacteriological identification by VITEK 2 system confirmed that the isolate was *E.coli*, with the VITEK 2 system achieving an identification accuracy ranging from 93% to 98%.

Table (3-1): Bacterial identification and Biochemical Tests

Cultural and biochemical tests	Results of *E.coli*
Gram stain	**(-)**
Lactose fermentation test on MacConkey agar	lactose fermenter
EMB agar	Green metallic sheen
Blood agar	Non hemolysis
oxidase test	**(-)**
Catalase test	**(+)**
Motility test	Motile
Citrate utilization test	Green color (-)
Urease test	No change (-)

Susceptibility of Antibiotic

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Antibiotic Susceptibility test was carried out using VITEK 2 compact, and the MIC-based results were interpreted according to EUCAST recommendations. Gram-negative *E. coli* susceptibility was tested before and after the exposure of *E.coli* to green tea leaf ethanol extract at 0.60, 0.50, 0.30, 0.16 and 0.08 g/mL concentrations.

The isolate was susceptible to multiple antimicrobial classes prior to exposure

After treatment with green tea leaf ethanol extract at concentrations of 0.16 and 0.08 g/mL, measurable changes in antibiotic susceptibility were observed.

According to globally recognized antimicrobial resistance definitions, the *E. coli* isolate satisfied the criteria of extensively drug-resistant (XDR) after exposure to both tested concentrations 0.16g/ml and 0.08g/ml of green tea extract because it was resistant to at least one agent in almost all antimicrobial categories examined. No bacterial growth was observed at concentrations of 0.30, 0.50, and 0.60 g/mL.

Resistance rates were also higher to β-lactam/β-lactamase inhibitor combinations (ampicillin/sulbactam, ceftolozane/tazobactam), carbapenems (imipenem, meropenem), aminoglycosides (amikacin, gentamicin) and trimethoprim/sulfamethoxazole combination of antifolate.as shown in Table (3.2).

In contrast, there were no alterations in the susceptibility pattern for tigecycline, cefepime, ceftazidime, cefotaxime, ciprofloxacin or colistin as the organisms after exposure to extracts remained resistant or susceptible. As shown in table (3.2).

Table (3.2): Antibiotic susceptibility profile of *Escherichia coli* before and after exposure to green tea leaf ethanol extract

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After 0.08g/ml	After 0.16g/ml	Before exposure	Antibiotic	Class
S	S	S	Tigecycline	Glycylcycline
S	R	I	Ampicllin /subactoram	B-Lactamase inhibiter
I	I	S	Ceftolozane /Tazobactam	
S	S	S	Ceftazidime / Avibactam	
S	R	S	Piperacillin /tazobactam	
R	R	R	Cefepime	Cephalosporins
R	R	R	Ceftazidime	
R	R	R	Cefotoxime	

R	**R**	**S**	**Imipenem**	**carbapenems**
R	**R**	**S**	**Meropenem**	
R	**R**	**S**	**Amikacin**	**Aminoglycosides**
R	**R**	**S**	**Gentamicin**	
R	**R**	**R**	**Ciprofloxacin**	**Fluoroquinolones**
I	**I**	**I**	**Colistin**	**Polymyxins**
R	**R**	**S**	**Trimethoprim / sulfamethoxazole**	**Antifolate antibiotic**
XDR	**XDR**	**Sensitive**	**Overall resistance pattern**	

R=Resistance I= intermediate S= Sensitive

- XDR classification was defined as resistance to at least one agent in nearly all tested antimicrobial categories, according to standard international definitions)Cosentino et al., 2023)
- XDR= R≥1 agent in (n−2) antimicrobial categories
- Where R denotes resistant isolates, n represents the total number of antimicrobial categories tested, and n − 2 indicates all antimicrobial categories except two at most

Discussion

As shown in table (3-2), the provided ethanolic green tea (*Camellia sinensis*) leaf extract in concentrations of 0.16 g/mL and 0.08 g/mL produced identifiable changes on antibiotic resistance profiles for *E.coli* isolates Several isolates exhibited multidrug resistance, and an in vitro extensively drug resistant (XDR) phenotype emerged. These results suggest that phytochemical-rich extracts of plant materials are capable of inducing substantial changes in susceptibility patterns of bacteria when applied under laboratory conditions.

Green tea is known to have possessed antimicrobial activity because of the presence of biologically active polyphenols, especially catechins like epigallocatechin gallate (EGCG). These agents are known to cause defects in the bacterial outer membranes, affect metabolic pathways and increase oxidative stress in microbial cells (De Rossi et al., 2025; Duda-Madej et al., 2025). But even though it has these antimicrobial effects, high concentrations or prolonged exposure to plant-derived compounds can also induce bacterial adaptive stress responses. This avoids the more common pattern of phenotypic resistance, which occurs when exposure to sub-inhibitory concentrations of phytochemicals increases susceptibility (Lima et al., 2023; Li et al., 2024).

In studies exploring the effects of green tea extracts on bacterial pathogens, similar observations have been reported (Mandal et al., 2024(. Green tea polyphenols have been found to affect the physiological state of *E.coli* by altering membrane integrity and interfering with metabolic processes. These compounds can disrupt cellular functions and induce oxidative stress, leading to physiological disturbances that may influence bacterial responses to antimicrobial agents and ultimately modify antibiotic susceptibility patterns under certain environmental conditions (Kiddee et al., 2024).

A possible explanation of the resistance shift observed in this study is that bacterial stress-response mechanisms have been activated. There are evidence that environmental stressors including phytochemical exposure activate regulatory systems that modify membrane permeability and multidrug efflux activity (De Rossi et al., 2025). Efflux pumps are one of the most significant contributors to antimicrobial resistance by actively pumping antibiotics out of bacterial cells, decreasing intracellular drug concentration and subsequently defeating antibiotic efficacy (Rouvier, F., et al., 2025).

In Gram-negative bacteria like *E. coli*, multidrug efflux systems including the AcrAB–TolC transporter are major contributors in facilitating resistance to multiple classes of antibiotics. It is a well-known adaptive reaction of bacteria because activation or up-regulation of such transport systems under stress conditions enable the macromolecules to survive in the presence of antimicrobial agents (Jang et al., 2023).

An additional mechanism is the change in outer membrane porin proteins. The porins OmpF and OmpC are channels that allow the hydrophilic antibiotics to enter Gram-negative bacterial cells. Thus, down-regulation or loss of these porin proteins may ultimately reduce membrane permeability and hinder the entry of antibiotics into the bacterial cell itself (Choi et al., 2025; Mmatli et al., 2025), which can lead to a higher resistance level in *E. coli* and related Enterobacterales.

Finally, one note must be highlighted that this study was not conducted on gene expression or molecular resistance determinants. Thus, their involvement of efflux pumps, porin regulation or sigma-factor-mediated stress responses should be understood as plausible models based on prior published works rather than experimentally substantiated mechanisms in the current study.

Notably, susceptibility to specific antibiotics like tigecycline and colistin remained relatively unaffected after exposure with green tea extract. This finding could be explained by the particular modes of action of these antibiotics. Tigecycline has a high affinity for the bacterial ribosome and is minimally impacted by common efflux-mediated resistance mechanisms, whereas colistin disrupts lipopolysaccharide structures on the outside of the bacterial membrane, which means that porin-mediated permeability changes have less of an impact on developing resistance (Bahaj et al., 2025).

In general, in the study presented here uncontrolled or overdose exposure of bacteria with plant extracts may inadvertently cause adaptive antibacterial resistance responses. The findings shed light on the need to assess possible interactions between herbal substances and antimicrobial agents, especially in cases where herbal compounds are administered together with standard antibiotic treatment.

Conclusion

The present study showed that treatment of *E.coli* clinical isolates with ethanolic extract of green tea (*Camellia sinensis*) leaf may affect the antibiotic susceptibility pattern of these isolates in vitro. Specifically, exposure to 0.16 g/mL and 0.08 g/mL concentrations increased resistance to several classes of antibiotics and led to the emergence of an extensively drug-resistant (XDR) phenotype in some isolates.

These results indicate that phytochemicals, coming from plants, may enter into interaction with bacterial cells and their sensitivity to antimicrobial agents in laboratory conditions. The mechanism(s) that led to those changes in the present study were not examined.

Limitations to this study include a relatively small number of clinical isolates evaluated as well as lack of molecular analysis to query mechanisms underlying the change in resistances observed. Thus, further investigations with larger sample sizes and molecular studies are necessary to delineate the mechanisms driving these findings and better characterize their potential relevance.

Ethical Approval

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Verbal informed consent was obtained from all participants prior to sample collection. The study protocol was reviewed and approved by the Ethics Committee of the College of Science, Department of Biology, University of Baghdad, Iraq (Approval No. CSEC/0825/0096; approved on 24 August 2025).

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