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The Effect of Alcoholic Extracts of *Zingiberofficinale* Anti-*E.Coli* Isolates Isolated from Urinary Tract Infection

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Abstract

The Escherichia coli isolated from UTIs, were identified with biochemical tests and vitek test.The ethanolic extracts of Zingiberofficinale(Z.officinale) were tested against E.coli by using the good agar diffusion test, the alcoholic extracts from (25 - 100) mg/ml showed antimicrobial activity against tested microorganism. The diameter of inhibition zone increase at high concentrations and rang from (18-20mm) , and these results compared with antibiotics sensitivity discs were used by discs diffusion method against E.coliisolates, they were resisted to all antibiotics used in this study. It could be concluded that alcoholic extract of Z.officinalehad good antimicrobial effects, and may be able to use for treatment UTIs caused by E.coli because it not make any side effect and can be used occasionally to prevent the infect by this bacteria.

Keywords: E.coli , Z.officinale , Antibiotics Resistance.

دراسة تأثير المستخلص الكحولي لنبات الزنجبيل على عزلات بكتريا الاشريكية القولونية المعزولة من التهاب المجاري البولية

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الخلاصة

عزلت بكتريا الاشريكية القولونية من التهاب المجاري البولية ,شخصت بالاختبارات الكيميائية الحيوية وبأستخدام نظام الفايتك , تم اختبار تأثير المستخلص الكحولي بتراكيز 25−100 ملغم/مل, حيث اظهرت التراكيز فعالية مايكروبية ضد البكتريا قيد الاختبار حيث زاد قطر منطقة التثبيط مع زيادة التركيز وتراوح 18−30 ملم وتم مقارنة هذه النتيجة مع نتيجة حساسية البكتريا لمضادات الحياة بأستخدام طريقة الانتشار حيث كانت البكتريا مقاومة لكل انواع المضادات المستخدمة في هذه الدراسة وتم التوصل الى انه المستخلص الكحولي لنبات الزنجبيل كانت له فعالية ضد مايكروبية جيدة ومن الممكن ان يستخدم لعلاج حالة التهاب المجاري البولية التي تسببها بكتريا الاشريكية القولونية وذلك لانه لايؤدي الى اي اضرار جانبية ومن الممكن ان يستخدم لمنع الاصابة بهذه البكتريا.

Introduction

In developing countries, urinary tract infections (UTIs) are one of the most common diseases among diverse age groups[1]. Females are more affected compared to males, UTIs can be categorized as acquired or nosocomial. E.coli is the most common organism responsible for UTI in both communities acquired and nosocomial and cause other infections such as diarrhea and bacteremia. E. coli can cause these infections because it has many virulence factors like adhesion, iron uptake, toxins, capsular polysaccharides , and proteins[2]. UTI caused by other bacteria like Klebsiella, Proteus, Pseudomonas, and Enterobacter. Increasing bacterial resistance to antibiotics has become the main concern due to

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misuse of antibiotics, sensitivity pattern should be made in UTI , So, it is necessary to look for the most sensitive antibiotics for proper treatment [3]. Z.officinale is a common natural herb and widely used as medicinal and food beverage purpose. The presence of pharmacological activities such as anti-inflammatory, anti-oxidatives and anti-cancer from the extraction of it mainly due to the existence of gingerols and shogaols[4].Z.officinalehasessential oils like udesmol, γ-terpinene, a-curcumene,alloaromadendrene,zingiberene (, α-pinene, δ-cadinene,elemol andfarnesal[5].The aim of this study is to determine the antimicrobial effectiveness of Z.officinale alcoholic extracts on E.coli isolates isolate from UTI and compare it with the effect of different antibiotics against this pathogen.

Materials and Methods:

Isolation of bacteria:

Fifty samples of urine were collected from Al-Yarmuk, and Al-Karama hospitals from patients (male amd female) suffering from UTIs.

biochemical tests:

The isolated bacteria were identified with morphological characters and biochemical tests.TheMacConkey agar (Himedia/India) was used for primary identification of E. coli and other gram negative bacteria; eosin methylene blue media used also in identification. Indole and Simmons citrate tests to identify E. coli from other Enterbacteriacae[6].The identification was confirmed by vitek system.

Antibiotics sensitivity test:

In vitro sensitivity test was done according to Kirby-Bauer [7]; after dilution to the standard turbidity of McFarland tube no. 0.5. With a sterile swab dip it into the broth culture of the organism and then were inoculated on Mueller Hinton agar plates. After the streaking is complete, allow the plate to dry for 5 minutes. Antibiotic discs can be placed on the top of agar by using sterilized forceps. The inoculated plates incubate over night at 37°C. In this study eleven antibiotics discs(Bioanalyse/Turkey) were used they are: Chlorotetracyclin (30µg), Sparfloxacin (10µg), Imipenem (10µg), Ticarcillin (10µg), Amoxicillin (5µg),Trimthoprim(30µg), Azithromycin (25µg), Cephradine (10 µg) and Ceftriaxon (15µg), Table-1, then measured the diameter of inhibition zone in millimeter by ruler for antibiotics used. Compare the measurement obtained with the standard table on NCCLS to determine the sensitivity zone [8].

Table 1-Antibiotics and their concentrations

Antibiotics	Concentration mg/ml
Chlorotetracyclin	**10**
Sparfloxacin	**10**
Imipenem	**10**
Ticarcillin	**10**
Amoxicillin	**30**
Trimethoprim	**20**
Azithromycin	**10**
Cephradine	**10**
Ceftriaxone	**15**

Preparations of plants extracts:

Dried powder of *Zingiberofficinale* was used in current study was bought from stores of plants in Baghdad city.

Soxhlet Extraction

The initial concentrations of bioactive compounds presented in the *Zingiberofficinale* matrix were identified through the soxhlet extraction. 25.0g of dried plant powder was extracted with 200 ml of 80% (v/v) aqueous ethanol by Soxhlet apparatus used for 8 hrs, then the extract was dried in oven with 40° C, and the power of plant extract was kept in a refrigerator (4) C^0 for further use [9], Figure-1.

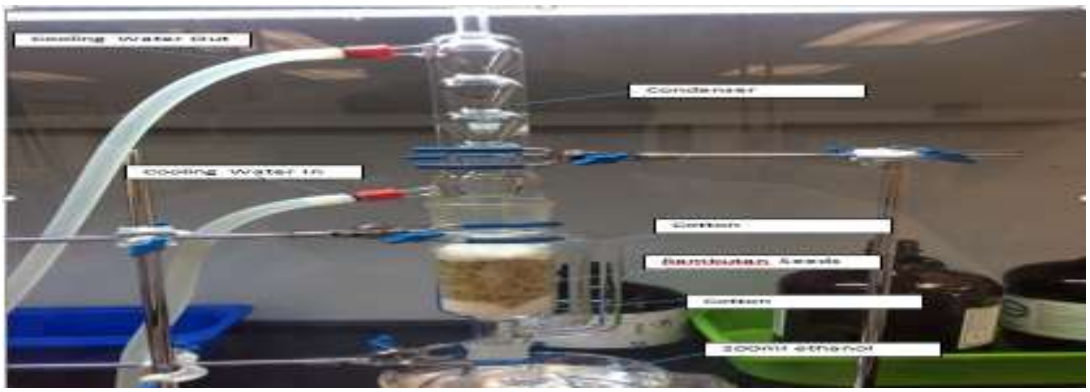

Figure 1-alcoholic extraction of *Zingiberofficinale*

Minimum inhibitory concentrations (MIC) determination of plants extracts:

The MIC of crude extracts was determined by broth macro-dilution assay. A set of test tubes with concentrations of plant extract (25,50,75 and 100) mg/ml. Tubes were inoculated with bacteria. After incubation, tubes were examined for changes in turbidity as an indicator of growth. The first test tube that appeared clear was considered as MIC of *Zingiber officinale* extract, against *E. coli* with used positive and negative control for comparison [9].

Well method

Plant extracts concentrations determined by well method to determine the presence of inhibition zones or not for*E.coli*isolates by made wells in the Mullar-hinton agar by cork borer after the spread of bacteria plates and adding the suspensions to the wells then incubated for 18 hrs. at 37C^{o}[10]

Result and discussion

Identification of bacteria by biochemical tests:

Fifty urine samples were collected from patients with UTIs. The samples were cultured on MacConkey agar, the bacteria gram negative appeared pink colonies because of lactose ferments in Table-2.

Table 2-the biochemical tests for E. coli identification:

The test name	Results
Microscopically	Gram negative
Indole	Positive
MR	Positive
VP	Negative
SimmonsCitrate utilization	Negative
MacConkey agar	Pink colonies
Blood agar	Alpha or gamma haemolysis
EMB agar	Green metallic sheen phenomena

Susceptibility of bacteria to antibiotics

The result showed that *E.coli* isolates had resistance to all antibiotics used in this study Table-3:

Table 3-Range of inhibition zoneby discs sensitivity test against *E. coli*:

Antibiotics discs	Range of inhibition zone diameter in mm of *E. coli* isolates	Percent of resistant isolates (%)
Chlorotetracyclin	0mm	100%
Sparfloxacin	0 mm	100%
Imipenem	0mm	100%
Ticarcillin	0mm	100%
Amoxicillin	0mm	100%
Trimethoprim	0mm	100%
Azithromycin	0mm	100%
Cephradine	0mm	100%
Ceftriaxone	0mm	100%
Chlorotetracyclin	0mm	100%

Table-3 shows *E. coli* which isolated from UTIs were resisted to all antibiotics used in this study, and this disagree with other studies that *E. coli* were resistant to commonly used antibiotics but sensitive to Nitrofurantoin, Amikacin and Cefotaxime are considered appropriate for empirical treatment of E. coli in the study area of [11]. Microorganisms causing UTI vary in their susceptibility to antimicrobials agents from region to others; while the extracts of plants were very active against these isolates.

Other study reported that the most effective antibiotic on *E.coli*was Trimethoprim [12]. Other study showed that lowest resistance of*E.coli* isolates isolated from UTI to ciprofloxacin, ceftizoxime[13]. This result may relate to misuse and random use of antibiotics which lead to high resistance to all antibiotics under testing.

The mechanisms of acquired resistance fall into one of the five categories, and these are 1)Enzymatic modification or destruction of the antibiotic, 2)Reduced antibiotic uptake into the bacterium, 3)Increased efflux of antibiotic from the bacterium, 4)Alteration or production of a new target site The mechanisms of acquired resistance fall into one of the five categories, and these are 1)Enzymatic modification or destruction of the antibiotic, 2)Reduced antibiotic uptake into the bacterium, 3)Increased efflux of antibiotic from the bacterium, 4)Alteration or production of a new target site The mechanisms of acquired resistance fall into one of the five categories, and these are 1)Enzymatic modification or destruction of the antibiotic, 2)Reduced antibiotic uptake into the bacterium, 3)Increased efflux of antibiotic from the bacterium, 4)Alteration or production of a new target site

The four main mechanisms by which bacteria exhibit resistance to antibiotics are drug inactivation or modification, Alteration of target- or binding site, Alteration of metabolic pathway and Reduced drug accumulation: by decreasing drug permeability or increasing active efflux (pumping out) of the drugs across the cell surface[14].

The mechanisms of acquired resistance fall into one of the five categories, and these are 1)Enzymatic modification or destruction of the antibiotic, 2)Reduced antibiotic uptake into the bacterium, 3)Increased efflux of antibiotic from the bacterium, 4)Alteration or production of a new target site

3- Effect of alcoholic extract of *zingiberofficinale* on bacterial isolated

The extract of *Zingiberofficinale* had high activity on*E.coli*isolates because there were inhibition zones which increase in size with the increasing of extract concentration as shown in Figure-2.

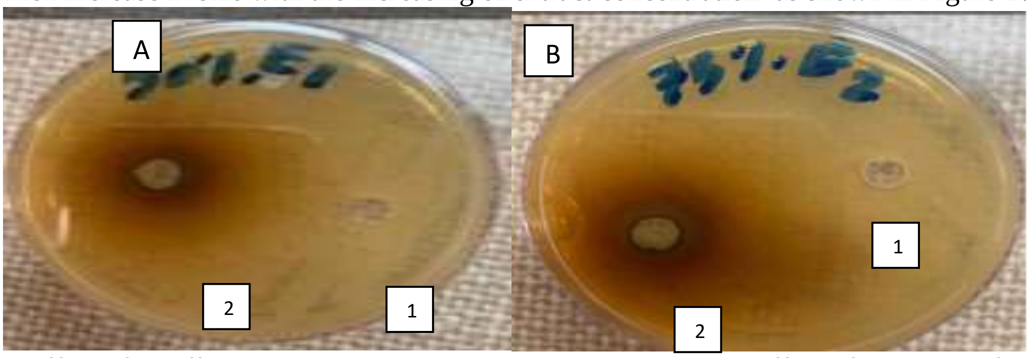

Figure 2-Effect of *Z.officinale* extracts on *E.coli* isolates E1 and E2A: Effect of 50mg/ml of plant extract on E1 , (1):control,(2):50mg/ml B:Effect of 75% of plant extract on E2,(1):control,(2):75mg/ml

Z.officinale had antibacterial activity to various bacteria like *E.coli* and *S.aureus* ,the zone of inhibition was 6 mm. [15].Other study showed that MIC concentrations range of *Z.officlnale* between 75 mg/ mL and 250 mg/ mL [16].The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids [17]. It is interesting to note that even crude extracts of these plants showed good activity against multidrug resistant strains where modern antibiotic therapy has limited effect.

Ginger is truly a world domestic remedy. It is also used in India and other places like the ancient Chinese where the fresh and dried roots were considered distinct medicinal products. Fresh ginger has been used for cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, less of appetite, and rheumatism, in short for the same purposes as in ancient china, In

nineteenth century ginger serves as a popular remedy for cough and asthma when the juice of fresh ginger was mixed with a little juice of fresh garlic and honey, A paste of powdered dried ginger was applied to the temples to relieve headache and fresh ginger was mixed with a little honey, tapped off with a pinch of burnt peacock feathers to alley nausea[18].

Conclusion

From the result, *E.coli* isolates which isolated from UTIs showed high resistance to antibiotics , this may be related to random use of antibiotics.

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