

Antimicrobial Potential of Various Essential Oils against Multidrug-Resistant Uropathogenic *Escherichia Coli*

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Abstract - Uropathogenic *Escherichia coli* is one of the major pathogens causing urinary tract infections. This study aimed to identify the prevalence of antibiotic resistance among pathogenic *E. coli* isolated from patients with urinary tract infections and evaluation of plant-derived essential oils as alternatives to antibiotics. The majority of isolated strains exhibited remarkable resistance against various antibiotics especially quinolones. Of 100 strains, 78 were found to acquire no susceptibility to at least one antibiotic in three or more classes and classified as multidrug resistant. The antibacterial potential of ten essential oils were investigated and results revealed that clove and oregano were the most powerful essential oils with MIC 0.25 and 1 µL/mL, respectively. The present results suggest that plant-derived EOs may assist in antimicrobial fight, especially those contain high amounts of eugenol and terpenoids (such as clove and oregano) which are active against multidrug-resistant strains.

Keywords - UPEC, Resistant, Quinolone, Antimicrobial

I. INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections that accounts for a severe morbidity and can lead to significant mortality. Uropathogenic *Escherichia coli* (UPEC) are responsible for 80–90% of community-acquired UTIs and causes bladder infections, acute kidney infections and infections of the bloodstream [1,2]. Many virulence factors contribute to the pathogenicity of UPEC and development of the infectious process, such as adhesins, toxins and siderophores [3–5]. UPEC attaches to epithelial cells of the urinary tract by type 1-fimbrial adhesins (*fim*), P-fimbrial adhesins (*pap*), afimbrial adhesins (*afa*) and S-fimbrial adhesins (*sfa*). Furthermore, capsule, hemolytic and cytotoxic necrotizing factors facilitate the invasion of the host and interfere with the host defense system [6].

Recently, the increased drug resistance spectrum of UPEC has become a major concern due to the emergence of

multi-drug resistant UPEC strains worldwide [7]. So novel antimicrobial strategies against the UPEC recently proposed have included the use of plant-derived essential oils. Essential oils (EOs) are aromatic oily plant extracts that characterized by a strong odor and produced by various plants as secondary metabolites that have shown good potential against drug-resistant pathogens [8,9]. The bioactivity of essential oil containing plants has been known since ancient times and widely used in traditional medicine. Plants such as lemongrass, oregano, neem, ginger, clove, rosemary, anise, thyme, lavender, and true cinnamon have been well-known to have various bioactivities, including antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant activities [10,11]. The present study is aimed to determine antibiotic resistance and investigate the antibacterial potential of ten EOs (*Cymbopogon citratus*, *Origanum vulgare*, *Azadirachta indica*, *Zingiber officinale*, *Syzygium aromaticum*, *Rosmarinus officinalis*, *Pimpinella*

anisum, *Thymus vulgaris*, *Lavandula spica*, and *Cinnamomum verum*) against multidrug-resistant UPEC strains.

II. MATERIAL AND METHODS

A. Isolation and identification of uropathogenic *E. coli*

Fresh midstream urine specimens were collected and cultured on MacConkey agar plates for 24 h at 37 °C. The typical purple colonies were then inoculated on Eosin Methylene Blue (EMB) agar plates and incubated for 24 h at 37 °C. Metallic green colonies were identified by standard microbiological and biochemical methods including citrate utilization, lactose and glucose fermentation in tubes with Kligler iron agar, urease and indole production [12].

B. Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by the Kirby-Bauer disk diffusion method using amikacin (30 µg), gentamicin (10 µg), cephalixin (10 µg), ciprofloxacin (5 µg), cephalothin (30 µg), cefixime (5 µg), cefpodoxime (10 µg), cephalozin (30 µg), cefepime (30 µg), tetracycline (30 µg), imipenem (10 µg), and vancomycin (30 µg). The resistance was determined according to the breakpoint proposed by CLSI [13]. Inocula were prepared by suspending bacterial colonies from overnight cultures in sterile saline to make the turbidity equivalent to 0.5 McFarland standards. Bacterial suspensions were spread onto Mueller Hinton agar then antibiotic disks were dispensed onto surfaces of the inoculated agar plates. After incubation period at 35 °C for 24 h, zones of inhibition were determined according to the CLSI recommendations. MDR strains were defined as having acquired no susceptibility to at least one antibiotic in three or more classes [14].

C. Essential oils

Lemongrass (*Cymbopogon citratus*), oregano (*Origanum vulgare*), neem (*Azadirachta indica*), ginger (*Zingiber officinale*), clove (*Syzygium aromaticum*), rosemary (*Rosmarinus officinalis*), anise (*Pimpinella anisum*), thyme (*Thymus vulgaris*), lavender (*Lavandula spica*) and true cinnamon (*Cinnamomum verum*) were kindly by the Essential Oil Unit (National Research Center, Giza, Egypt). The investigated essential oils did not contain additives or solvents and were stored in brown vials at 4 °C in the dark.

D. Antimicrobial activity of essential oils

Antibacterial activity of each essential oil was evaluated by the disk diffusion method. Briefly, bacterial suspensions equivalent to 0.5 McFarland standard were spread onto the

Mueller Hinton plates and sterilized discs (6 mm diameter) were impregnated with 10 µL of EOs and placed on the agar surface under aseptic conditions, and then were incubated at 37 °C for 24 h. Paper disc impregnated with sterile water was used as a negative control. After the incubation period, the zones of inhibition were measured and recorded.

E. Determination of minimum inhibitory concentration (MIC) of the most potent essential oils

The minimum inhibitory concentrations (MIC) were determined in 96-well plates as described before [15]. The final concentrations of essential oils were 500, 250, 125, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 µL/mL. The MIC value was determined as the lowest concentration of the EO that inhibited bacterial growth.

III. RESULTS

A. Isolation and identification of uropathogenic *E. coli*

One hundred UPEC strains isolated from midstream urine specimens were identified according to biochemical and phenotypic properties. Bacterial strains that showed metallic green colonies on EMB were collected and subjected to further investigations. Gram-negative lactose fermenting bacteria that have negative response to urease, citrate utilization and positive response to indole test were identified as *E. coli*.

B. Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed against different antibiotics belonging to various classes by the disk diffusion method and the antibiogram was presented (Fig. 1). The UPEC strains showed the highest rates of susceptibility to cefixime (29%), followed by imipenem (28%), and ciprofloxacin (24%). In contrast, lowest susceptibility rates were observed for cefepime (100%), tetracycline (98%), and cephalothin (83%). Of 100 UPEC strains, 78 acquired no susceptibility to at least one antibiotic in three or more classes and classified as MDR.

C. Antimicrobial activity of essential oils

Antibacterial activity of each essential oil against UPEC strains was evaluated by the disk diffusion method. Only 5 EOs exhibited antimicrobial activity against the investigated strains (Fig. 2). All strains were resistant to essential oils derived from lemongrass, neem, ginger, and lavender, on the other hand, clove EO was active against 100% of the investigated strains. Based on these results, four EOs (oregano, clove, rosemary, and true cinnamon) were selected as potent antimicrobial agents against UPEC.

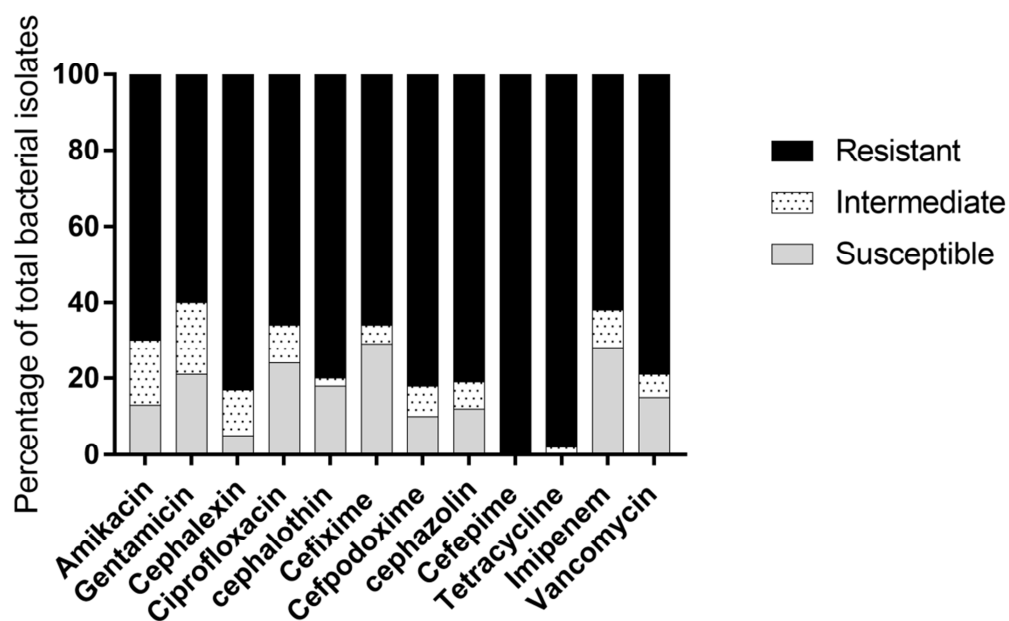


Figure 1: Antibiotics resistance profile of uropathogenic *E. coli*

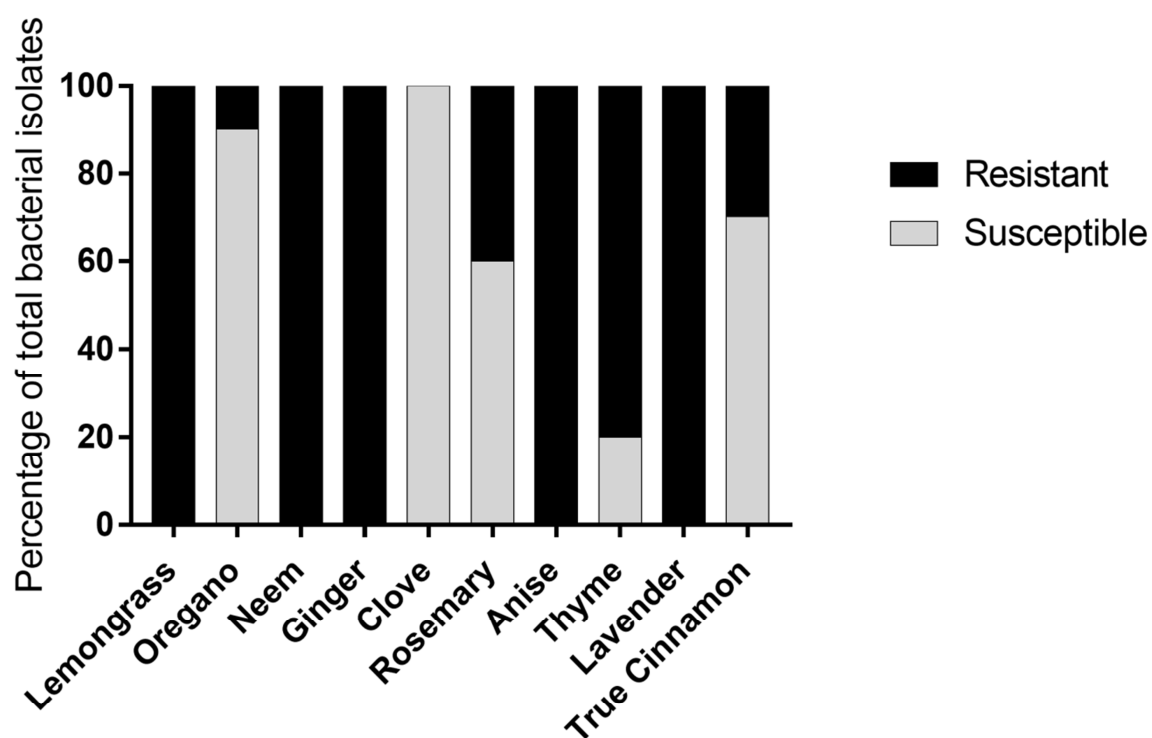


Figure 2: Antimicrobial activity of various essential oils against uropathogenic *E. coli*

A. Minimum inhibitory concentration (MIC) of the most potent essential oils

The MIC values of oregano, clove, rosemary, and true cinnamon were determined in 96-well plates against 10 MDR UPEC strains that exhibited resistance to the most of the investigated antibiotics. The superior antimicrobial activity was observed by clove EO that showed an inhibitory effect against MDR strains at 0.25 $\mu\text{L/mL}$. Next to clove, oregano demonstrated a potent antibacterial effect with MIC value of 1 $\mu\text{L/mL}$ while the least inhibitory effect was recorded by rosemary and true cinnamon (Table 1).

Table 1: MIC of the most potent essential oils against multidrug-resistant UPEC

Essential Oil	MIC ($\mu\text{L/mL}$)
Oregano	1
Clove	0.25
Rosemary	4
True Cinnamon	4

IV. DISCUSSION

The emergence of antibiotic resistance and MDR-phenotype in high rates from urinary tract infections becomes a public health concern worldwide. In this study, 78% of the isolates showed resistance to at least one antibiotic in three or more classes and classified as MDR. The majority of the investigated UPEC strains were resistant to quinolones which are recommended as a first choice for UTI treatment. The prevalence of multidrug-resistant strains in this work was higher than in other study which reported only 16.4% of MDR strains in Mexico [16]. Ciprofloxacin is the most common fluoroquinolone used to treat UTIs. However, due to its overuse, the resistance rate of UPECs to that antibiotic has markedly increased in the last decade [17]. Furthermore, the use of antibiotics in livestock for growth promotion is suggested to be another important factor contributing to antimicrobial resistance [18]. Our results revealed that clove and oregano EOs were the most active agents compared with other assessed EOs. The MIC was 0.25 and 1 $\mu\text{L/mL}$ for clove and oregano EOs, respectively. Similar results were reported about the powerful antimicrobial activity of clove and oregano EO against *E. coli* and other pathogens [19–23]. It has been suggested that eugenol, the main component, is thought to be responsible for the strong biological and antimicrobial properties of clove EO [24]. Eugenol has also been shown to cause deterioration of the cell wall, lysis of cells, and

prevention of enzyme action [25]. Oregano is a popular herb contains essential oil has been used in folk medicine since ancient times. The main component of oregano EO is carvacrol, a monoterpenoid phenol, which can present antioxidant and antibacterial activity [26,27].

V. CONCLUSION

The present results suggest that plant-derived EOs may assist in antimicrobial fight, especially those contain high amounts of eugenol and terpenoids (such as clove and oregano) which are active against multidrug-resistant UPEC.

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