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Prospective antibacterial behavior of some essential aromatic oils against new *Escherichia coli* strain of extended-spectrum Beta-lactam

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Abstract

Aim: To evaluate the antimicrobial activities of some aromatic essential oils against a new pathogenic *Escherichia coli* strain with extended-spectrum beta-lactamase (ESBL) activity.

Methodology: A total of 66 enteric bacteria were isolated from 250 urine samples that had been collected from human patients at different hospitals in Cairo, Egypt. Virulence factors of pathogenic isolates were determined, and the antibacterial activities of cinnamon bark oil and clove oil against *E. coli* EMH-542017 were estimated using the VITEK 2 system; this strain was confirmed as *E. coli* based on its 16S rRNA gene sequence, which was deposited in NCBI GenBank. Finally, modification of the bacterial cell wall following treatment with essential oil was observed with transmission electron microscopy (TEM).

Results: The results confirmed that *E. coli* EMH-542017 was capable of producing hydroxamate siderophores and was resistant to 11 antibiotics, which were significantly observed with different grades. Nevertheless, screening by the combination disc method showed that 20 isolates were ESBL producers. Polymerase chain reaction analysis confirmed that the selected isolate EMH-542017 was positive for both ESBL blaCTX-M-9 genes. The addition of cinnamon and clove oils at levels of 12.5, 25, and 50 mg ml⁻¹ was revealed to have high bactericidal effects on bacterial growth, and a change in bacterial cell morphology was detected by TEM, in which the cell wall seemed to be damaged and the flagella were lost following essential oil treatments, demonstrating that essential oil treatments are an effective means of suppressing multidrug-resistant *E. coli*.

Interpretation: Despite these results, there is an urgent need to identify effective, safe, and economical treatments to reduce and protect against urinary tract infections in Egyptian patients.

Key words: Cinnamon bark oil, Clove oil, *Escherichia coli*, Extended-Spectrum Beta-Lactamase, Urinary tract infections

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Introduction

Urinary tract infections (UTIs) in developing countries have received a considerable amount of attention. UTIs are typically difficult to eradicate, as most pathogenic bacteria are resistant to commonly used antibiotics. UTIs cause significant morbidity in females, infants, elderly males, people with inherent defects in their urinary tracts or bladders, and those with renal cell carcinoma (Busha, 2018; Hassan *et al.*, 2018). *Escherichia coli* is one of the main causes of nosocomial and communicable human diseases. The most appropriate therapeutic choices for treating diseases caused by *E. coli* are β -lactam antibiotics (primarily extended-spectrum cephalosporins and carbapenems) and fluoroquinolones (Busha, 2018; Rahman *et al.*, 2018), which revealed that *E. coli* strains could produce both AmpC β -lactamase and ESBLs. Furthermore, AmpC β -lactamase are clinically concerning cephalosporinases that confer resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and β -lactamase inhibitor- β -lactam combinations; genes for AmpC β -lactamases occur on the chromosomes of many Enterobacteriaceae bacteria and in few other microbes (Hassan *et al.*, 2018). The main mechanism for the action of β -lactam antibiotics on Gram-negative bacteria is to generate β -lactamase enzymes, which move via genetic-like plasmids by these enzymes (Hassan *et al.*, 2018). Approximately, 70%–90% of all diseases in the urinary tract are triggered by uro-pathogenic *E. coli* strains (UPECs), and pathogenesis involves contact between the bacteria and urinary tract surface due to major resistance determinant for beta-lactam antibiotics in Gram-negative bacteria (Busha, 2018; Ganz, 2018).

Iron is important for all living cells and *E. coli* uses iron for oxygen retention and storage, DNA synthesis, electron transport, and metabolism of peroxidase, which enables pathogenic *E. coli* to persist and survive in the bloodstream and other human tissues (Ganz, 2018). Bacteria that secrete siderophores as vital organic compounds for chelating iron may have a growth advantage under low-iron conditions. Rahman *et al.* (2018) demonstrated that the ability of non-pathogenic *E. coli* to synthesize virulence-associated siderophores may also enhance their ability to survive in external environments outside human hosts. Berendsen *et al.* (2020) reported that siderophores or aerobactin are essential for the survival of invasive strains of *E. coli*, where utilization of host iron sources such as transferrin or hemoglobin may provide an alternative method of iron acquisition.

In fact, the majority of enterobacterial infections caused by ESBL-producing organisms are *E. coli* and *K. pneumoniae* strains carrying CTX-M gene responsible for community infections, especially UTIs (Moubareck *et al.*, 2005; Alzahrani *et al.*, 2016). The SHV family of β -lactamases appears to be derived from *Klebsiella* spp. TEM, first reported from an *E. coli* isolate in 1965, has substrate and inhibition profiles similar to those of CTX genes. CTX is a new family of β -lactamases that preferentially hydrolyze cefotaxime. It has been found in isolates of *Salmonella*

enterica serovar, *Typhimurium*, *E. coli*, and some other species of Enterobacteriaceae (Moubareck *et al.*, 2005; Alzahrani *et al.*, 2016; Hassan *et al.*, 2018).

Several studies have indicated that essential oils are effective in addressing various medical issues, including the crisis of antibiotic resistance by infectious bacteria (Hassan *et al.*, 2014; Chouhan *et al.*, 2017). In general, the antibacterial effects of essential oils, such as cinnamon bark and clove oils, and their components are due to membrane and metabolic effects on *E. coli*, as well as medicinal properties (Kelothe *et al.*, 2018; Berendsen *et al.*, 2020). The health advantages of clove oil include its antimicrobial, antifungal, antiseptic, antiviral, and aphrodisiac characteristics (Berendsen *et al.*, 2020). Phenylpropanoids, such as carvacrol, thymol, and eugenol, are the principal components of oil (Packyanathan and Prakasam, 2017). In this study, a new Egyptian *E. coli* isolate was isolated, identified and assessed for its various virulence factors and their impacts on drug resistance. In addition, the *in vitro* antibacterial properties of cinnamon and clove oils against this new pathogenic bacterial strain was also investigated.

Materials and Methods

Isolation of *E. coli* bacterium: Sixty-six clinical *E. coli* isolates were isolated from 250 urine samples from different hospitals in Cairo, Egypt and cultured on tryptone soy agar medium. A standard bacterial inoculum was prepared according to the methods set forth by the Clinical and Laboratory Standards Institute (CLSI, 2015).

Screening of *E. coli* isolates for antibiotic resistance: *E. coli* isolates were assessed for antibiotic resistance by disk diffusion method and Kirby Bauer technique (CLSI, 2015). The antibiotic disks were obtained from Himedia Co., India, and cephalothin (CL, 30 μ g), cefuroxime (CXM, 30 μ g), co-amoxiclav (AMC, 20/10 μ g), cefotaxime (CTX, 30 μ g), ceftazidime (CAZ, 30 μ g), ceftriaxone (CRO, 30 μ g), cefepime (FEP, 30 μ g), imipenem (IMP, 10 μ g), gentamicin (CN, 10 μ g), ciprofloxacin (CIP, 5 μ g), and co-trimoxazole (SXT, 23.75 μ g) were applied. The diameters of inhibition zones were then measured.

Detection of siderophore production: Chrome Azurol-S (CAS) agar medium (Schwyn and Neilands, 1987) was used for the qualitative detection of siderophore production by *E. coli* isolates. The CAS reaction rate was determined by measuring the advance of color-change front in the CAS-blue agar, starting from the borderline. The CAS-agar color changed from blue to orange, purple or dark purplish-red (magenta).

Production of blood hemolysis: Blood hemolysis was detected qualitatively with or without 5% sheep blood agar (Oxoid, UK) after incubation for 24 hr at 37 °C, as previously described by Mac-Faddin (2000); Aljanaby and Alfaham (2017).

Screening for ESBL production by combination disk

method: Screening was performed according to CLSI (2015) standards, using ESBL detection kits (Himedia, Co., India), cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg), and ceftazidime/clavulanic acid (30/10 µg). The test organism was regarded as an ESBL producer if the inhibition zone formed around the combination disc was at least 5 mm larger than that of cephalosporin alone, or if the zone diameter expanded by 50% in the presence of clavulanic acid (CLSI, 2015).

Identification of *E. coli*: The selected *E. coli* EMH-542017 isolate was identified based on standard biochemical tests by (VITEK2 system version: 07.01) and confirmed by 16S rDNA sequencing. The genomic DNA was isolated by DNA extraction kit (Gena Bioscience, Germany) from *E. coli* EMH-542017 isolate as per manufacturer's instructions. The 16S rRNA of new isolate was amplified by thermocycler (Biometrathermocycler, Germany) using universal primers; Bact 27F, 5'-AGAGTTTGATCCTG GCTCAG-3'; 1492R, 5'-GGTACCTTGTTACGACTT-3 (Chang et al. 2000). The specific band was punctuated by QIA quick PCR purification kit (QIAGEN, Valencia, CA, USA) and sequenced in DNA Analyzer 3146 (Applied Biosystems, USA). The *E. coli* 16S rDNA sequences obtained were compared with known 16S rDNA sequences in GenBank using BLAST on the NCBI website (<http://www.ncbi.nlm.nih.gov/blast/>), and a phylogenetic tree was constructed using MEGA program version 7.10.

Detection of ESBL genes: The *E. coli* EMH-542017 isolate was tested for two types of CTX-M genes by PCR and sequencing assays described by Hassan et al. (2018). Primer sets developed by Pitout et al. (2009) targeting the group I and IV blaCTX-M genes were used. Sequence processing was performed as described above.

Screening of essential oils for antibacterial activity against selected ESBL-producing *E. coli* isolates: Disk diffusion method (Deans and Ritchie, 1987) was followed to evaluate the antibacterial efficiency of selected commercial essential oils. Caraway, cinnamon bark, clove, fennel and peppermint oils with concentrations of approximately 95% were obtained from different pharmaceutical sources. After incubation for 24 hr, the diameter of inhibition zones were measured and documented according to the method of Swenson and Thornsberry, (1984).

Growth curve technique and kill time for new strain EMH-542017: A typical inoculum of approximately 10^5 CFU/ml¹ was incubated at 37°C with agitation (200 rpm). The test concentrations was 2, 4, 6, 12.5, 25 and 50 mg ml⁻¹ TBS medium was used as control with or without added the ESBL-producing *E. coli*; control + Tween (without treatment; with 0.5% Tween 80); and a growing flask containing 10 ml of final volume. Optical density was measured at 600 nm for 100 µl of samples every 3 hr until 24, 36 and 48 hrs immediately after incubation. Ten-fold serial dilutions of 0.9% (w/v) sodium chloride were used. The analysis was carried out according to CLSI, (2009), and the

experiment was performed in triplicate.

Specimen preparation for scanning electron microscopy (SEM): The morphology of *E. coli* EMH-542017 ESBL-producing cells was examined by scanning electron microscopy (SEM) before and post treatment with essential oils following previously described methods (Hassan et al., 2014). Bacterial samples, collected before and post essential oil treatments, were fixed for 24 hr with vapors of glutaraldehyde and osmium tetroxide (3:1), air-dried for 48 hr, and then coated with gold.

Statistical analyses: Statistical analysis was done by ANOVA, F-test and LSD procedures available within the SAS software package version 9.1.3 (SAS2007). All data were subjected to Duncan's Multiple Range Test (Duncan, 1955) to discriminate significance (defined as $p < 0.05$).

Results and Discussion

Urinary tract is one of the most common sites for bacterial infection, and *E. coli* is the most common UTI infectant, which can cause acute, chronic and persistent diseases (Rahman et al., 2018; Hassan et al., 2018). In the current study, we demonstrated that 20 of the 66 *E. coli* isolates were ESBL producers (Fig. 1). An ESBL-producing phenotype is confirmed for an isolate if the difference between its zone diameters on the cephalosporin disk and its respective cephalosporin/clavulanate disk is ≥ 5 mm (Paterson and Bonomo 2005; Hassan et al., 2016). The use of ceftazidime on its own led to the discovery of organisms that produce the CTX-M gene (Hassan et al., 2018). In *E. coli*, the hydroxymate siderophore produced is called aerobactin (Hassan et al., 2014). Siderophores play an important role as virulence factors that contribute to bacterial growth in fluids (including serum and dilute urine) and host tissues (Aljanaby and Alfaham, 2017).

CLSI recommends the use of cefotaxime (30 µg) or ceftazidime disks (30 µg) with or without clavulanate (10 µg) to confirm ESBL production by *E. coli*. It further recommends that clavulanic acid solution should be applied to the cephalosporin disks not more than an hour before they are used in ESBL assays on agar plates. An ESBL-producing phenotype was confirmed if the difference in the diameters of its zones of production on cephalosporin disk and its respective cephalosporin/clavulanate disk exceeded 5 mm. The use of ceftazidime alone has led to the detection of organisms that produce CTX-M gene.

The antibacterial activities of *E. coli* isolates and the pattern of antibiotic susceptibility among the *E. coli* isolates from human urine samples are shown in Table 1. It was found that 100% of isolates were resistant to cefuroxime, ceftazidime, cefotaxime amoxicillin/clavulanate, ceftriaxone, cefepime and cephalothin. Moreover, isolates were found to be strongly resistant to gentamicin (60%), ciprofloxacin (80%), and trimethoprim-sulfamethoxazole (95%). The highest sensitivity

Fig. 1 : Screening of essential oils for antibacterial activity against selected ESBL producing *E. coli* EMH-542017 isolate.

Table 1: Antimicrobial susceptibility test of twenty *E. coli* isolates

Antibiotics / Disk concentration	ESBL producers (n=20)		Resistance (%)
	Sensitive	Resistant	
Imipenem (IMP) / 10µg	20 ^a	0	0
Gentamicin (CN) / 10µg	8 ^c	12 ^c	60
Ciprofloxacin (CIP) / 5µg	4 ^d	16 ^b	80
Trimethoprim-sulphamethoxazole(SXT)/1.25/23.75µg	1 ^d	19 ^a	95
Cefuroxime (CXM) / 30µg	0	20 ^a	100
Ceftazidime(CAZ) / 30µg	0	20 ^a	100
Cefotaxime (CTX) / 30µg	0	20 ^a	100
Amoxicillin/clavulanate(2:1)(AMC)/20/10µg	0	20 ^a	100
Ceftriaxone (CRO) / 30µg	0	20 ^a	100
Cefepime (FEP) / 30µg	0	20 ^a	100
Cephalothin (CL)	0	20 ^a	100

Means in the same column not followed by same letter differ significantly at 5% level

(100%) of the isolates was recorded for imipenem. Beta-lactamase is currently the main protection against beta-lactam antibiotics for Gram-negative bacteria; these enzymes break the amide bond in the beta-lactam ring of penicillin group of drugs. All 20 isolates examined in this study showed multiple resistances to at least seven antibiotics (2 B).

The antibacterial activity of five essential oils for ESBL generating of EMH-542017 was presented in Fig (1), which clarified isolate EMH-542017 was significantly highly sensitive to

all tested essential oils. Cinnamon bark oil exhibited the strongest antibacterial activity against (25 mm); clove oil 18 mm, while caraway oil exhibited moderate activity (10 mm). Both peppermint and fennel oil had similar and comparative activities against the tested isolate had been shown only 7mm. Cinnamon bark and clove oil were selected as the most active essential oils for further experiments. Abdel-Moaty *et al.* (2016) and Caceres *et al.* (2020) elucidated that the major components cinnamon oil are cinnamaldehyde, cinnamyl acetate, and clove oil are eugenol and α -caryophyllene interact with proteins and have the capability to

inhibit permeability of the cell membrane of against ESBL producing *E. coli* beside EOs significantly inhibited the biofilm formation in different pathogenic bacteria.

The qualitative assay (Fig. 2 C) confirmed that the new isolate had the ability to produce and secrete hydroxymate siderophores. The host response to infection is to decrease the amount of iron accessible to the invading pathogen by reducing the absorption of intestinal iron, synthesizing additional iron proteins, and transferring iron from the plasma pool to intracellular storage. Ferric iron is therefore highly insoluble, resulting in a low free iron concentration at pH 7. During infection, bacteria face a formidable challenge in fulfilling their iron needs. In *E. coli*, the hydroxymate siderophore aerobactin is most powerful of several iron chelation systems used for the acquisition of iron by enteric bacteria.

Antimicrobial resistance in *E. coli* is an extraordinary concern worldwide because of its increasing resistance to several commonly prescribed antibiotics (Hassan *et al.*, 2018). In the current study, *E. coli* isolates fluctuated in their susceptibility to different antibiotics belonging to different groups and were resistant to most tested antibiotics. These results are comparable to those of other local and global studies (Hassan *et al.*, 2014). The high level of resistance observed for certain antibiotics might be due to the spontaneous and uncontrollable use of these

antibiotics (Yang *et al.*, 2020; Sana *et al.*, 2016). In contrast, the carbapenems (imipenem and meropenem) are known to be stable against ESBL enzymes and effective in the treatment of infections caused by ESBL-producing bacteria, and the administration routes of these antibiotics (either intravenous or intramuscular) limit their use by most patients (Marialouis and Santhanam, 2016; Busha, 2018).

In the ESBL confirmatory test, *E. coli* isolates that were resistant to at least CAZ, CRO, and/or CTX were tested on CAZ and CTX disks and ceftazidime plus clavulanic acid (CAZ+C) and cefotaxime plus clavulanic acid (CTX+C) combination disks, which confirmed these as positive ESBL isolates (Abdel-Moaty *et al.*, 2016). Frequent irrational use of antibiotics has resulted in the development of bacterial strains with antibiotic resistance. Otherwise, β -lactam antibiotics may interfere with bacterial cell wall synthesis and kill susceptible bacteria (Berendsen *et al.*, 2020). Bacteria that produce ESBLs are capable of swapping ESBL genes between and within species (Mukherjee *et al.*, 2013; Hassan *et al.*, 2018). Lactamases that are able to hydrolyze approved cephalosporins, monobactams, and carbapenems have become prevalent among the Gram-negative pathogens (Busha, 2018). A reciprocal relationship was found between the bacterial secretion of siderophores and hemolysin, which lyses host blood cells and releases intracellular iron complexes in blood isolates (Busha, 2018). Hemolysin production is a mechanism by

Fig. 2 : Detection of virulence factors in new strain *E. coli* EMH-542017, where A: Combined disk assay; B: Secreting of hydroxymate siderophores; C: Antibiotics resistance; D: Gamma blood hemolysis; E: Growth on MacConkey and F: Examination of bacterial cell by Scanning electron microscope (5μm).

which microbes produce iron for bacterial growth and virulence factors in *Vibrio* sp. (Tay *et al.*, 1995; Hassan *et al.*, 2014).

The new isolate was considered γ -hemolysin producers if no hemolysis was observed on agar containing human blood (5%). (2D). Furthermore, a reciprocal relationship was found in blood isolates between the siderophore secretion ability and hemolysin, which lyses host cells and releases intracellular iron complexes. Siderophores, as iron transport systems, may be important and involved in the virulence of a variety of bacterial pathogens.

The selected ESBL-producing isolate EMH-542017 was examined by PCR for the presence of ESBL genes. Based on PCR analysis, the new *E. coli* isolate was found to be a producer of the blaCTX-M-IV enzyme. Fig. 3 shows the PCR assay results for the isolates, in which 98% of the tested isolates harbored CTX-M-type ESBLs of either subgroup CTX-M-I. Sequencing the PCR products amplified from the selected isolate that was positive for the presence of CTX-M-I genes was performed in order to identify

to which group of the CTX-M family their CTX-M-I genes belonged. DNA sequence analysis of the whole CTX-M-I gene from the isolate EMH-542017 revealed it had 98% similarity to the blaCTX-M gene.

The 16S rRNA gene was successfully amplified and sequenced from the 12 clinical bacterial isolates. Individual BLAST searches for each of the 16S rRNA sequences confirmed that they were multidrug resistant strains of *E. coli*. These results agree with those of Hassan and Belal (2016), who reported that the traditional identification of bacteria using phenotypic characteristics is generally not as accurate as identification by genotypic methods. Moreover, DNA sequence analysis of the whole CTX-M-I gene from the isolate EMH-542017 revealed that it was 98% similar to the blaCTX-M gene.

The characterization of virulence genes can improve our understanding of the pathogenesis of diseases and minimize the resulting complications. All bacterial strains in the current study contained at least one virulence gene (Paterson and Bonomo,

Fig. 3 : Electrophoresis gel showing PCR amplification products of bla CTX-M gene in *E. coli* isolate EMH-542017. Lanes from left to right; first lane : DNA ladder 10bands; 100 bp to 1000 bp, second lane: amplicon with 494 bp.

Fig. 4 : PCR amplification fragment of 16S rRNA gene for *E. coli* EMH-542017. Lanes from left to right; first lane: DNA ladder, second lane: amplicon with 1465 bp.

Fig. 5 : Neighbor-joining phylogeny based on 16S rRNA gene sequences of *E. coli* EMH-542017 isolates and related *E. coli* isolates obtained from a BLAST search of the NCBI database.

2005). The ESBL-encoding genes are found on mobile elements (plasmids or transposons), but also on the bacterial chromosome (Saliu et al., 2017). Beta-lactam antibiotics are often considered the last treatment available for serious, multi-drug resistant infections in humans (Hassan and Belal, 2016). Because of their role in human infections and their potential to harbor antimicrobial resistance, it is important to identify drug resistant strains of *E. coli*. This has proven effective in clinical epidemiologic studies; however, genetic heterogeneity has been previously described for *E. coli* strains from hospital patients and environmental sources (Hassan et al., 2014).

The ESBL-encoding genes are usually found on mobile elements (plasmids or transposons), but has also been observed on the bacterial chromosome (Saliu et al., 2017). ESBL genes are distributed vertically through cell division or gene transfer within each species and horizontally through conjugation with other organisms and genes. Sütterlin et al., (2014) reported that The types of ESBLs in humans are CTX-M-15, CTX-M-14 and CTX-TEM. ESBLs are characterized by their ability to hydrolyze beta-lactam rings and their susceptibility to β -lactamase inhibitors, such as clavulanate and extended-spectrum cephalosporins. However, they do not hydrolyze cephamycin. β -lactamases are therapeutic strategies to fight β -lactamasic development and development to increase β -lactams that are capable of resisting hydrolysis and the use of high-affinity β -lactamase inhibitors, known as suicide substrates (Hassan and Belal, 2016). Although beta-lactams are defined as inhibitor-resistant proteins, all these drugs effectively inhibit β -lactamases, including ESBLs. These results corroborates with the previous study of Trajano et al. (2010) who reported that the essential oil of *Cinnamom zeylanicum* as an alternative source of antimicrobial compound was effective against *Staphylococcus aureus* in food protection systems. Cinnamon bark oil is the most potent essential oil

against bacterial ESBL strains. The results we obtained for *E. coli* EMH-542017 in the time-kill assay following the cinnamon bark oil treatment is in line with those of Keloth et al. (2018), who confirmed that cinnamon bark oil has a remarkable potential to inhibit the growth of *E. coli* strains with ESBLs in UTIs.

The results showed that the best concentration of cinnamon bark oil was 6 mg ml⁻¹, and increasing the concentration with the two-fold technique led to cell death. Cinnamon bark oil 12.5, 25, and 50 mg ml⁻¹ caused cell death as evidenced by lack of growth across all isolates after 24 hr of incubation (Fig. 7), a finding that is consistent with those of previous studies (Abdel-Moaty et al., 2016; Keloth et al., 2018). These impacts are ascribed to the lipophilic nature and low molecular weight of important elements consisting of essential oils that enable them to cross cell membranes, change membrane structure, and boost membrane fluidity, resulting in ion leakage and cytoplasmic molecules.

The culture apparatus suggested that *E. coli* isolate (Fig. 2E) produced lactose-fermenting, non-swarming colonies on the MAC plate. Morphological examinations of the cells were conducted using SEM (Fig. 2F), and the cells were characterized as non-spore forming short bacilli with peritrichous flagella. VITEK 2 microbial identification system includes an expanded identification database as bio-number was 0405610544544610 with identification level with excellent 99% probability was used for the automated identification of new isolate. Seventeen out of 64 biochemical tests yielded positive results with beta-galactosidase (BGAL), glucose (dGLU), fermentation glucose (OFF), D-maltose (dMAL), D-mannitol (dMAN), D-mannose (dMNE), tyrosine arylamidase (TryA), D-sorbitol (dSOR), trehalose (dTRE), 5-Keto d-gluconate (5KG), L-lactatealkalinization (iLAKT), succinate alkalinization (SUCT), phosphatase (PHOS), lysine decarboxylase (LDC), coumarate (CMT), beta-glucuronidase (BGUR), and O-129-resistance (O129R). The 16S rRNA gene amplicon (~1.9 Kbp) from the local isolate, *E. coli* EMH-452017, was resolved by agarose gel electrophoresis (Fig. 4). The partial sequences of 16S rRNA gene of new Egyptian strain were deposited in the GenBank database under accession number MN960309, and the 16S rDNA nucleotide sequences revealed 100% genetic similarity (Fig. 5). When these sequences were aligned with the database sequences. The phylogenetic relationships among the studied *E. coli* strain based on comparisons of their partial sequences were inferred by neighbor-joining method. Phylogenetic analysis revealed 100% similarity between *E. coli* EMH-542017, *E. coli* CP054343, *E. coli* KY450353, *E. coli* MW582672, *E. coli* CP054232 and *E. coli* KY780358 that infect humans. PCR technique is a specific, simple, sensitive, and effective molecular technique for identifying *E. coli* infection (Hassan et al., 2018).

Optical control density values increased considerably and rapidly, suggesting that *E. coli* treated with the essential oils

Fig. 6 : Effect of different concentrations of cinnamon bark oil and clove oil on the growth of new isolate *E. coli* EMH-542017, A: Cinnamon bark oil and B: Clove oil.

for 12 hr reached the logarithmic process, in contrast to the growth curves observed for other therapies (Fig. 6). The new strain *E. coli* EMH-542017 was inoculated into medium with a 1:5 ratio of TSB to Tween and then incubated at 37 °C during the growth stages and checked for the activities of cinnamon bark oil and clove oil (Fig.6). The exponential development stage began at the 3rd hr from the beginning of incubation and continued until 12th hr of bacterial growth. The optical density increased to 600 nm (from 0.55 nm to 0.563 nm) during this stage, and the impact of

cinnamon bark oil and clove oil began at 3rd hr, which is the beginning of the exponential phase. The antimicrobial impact of cinnamon bark oil and clove oil began early at logarithmic phase (3rd h), and the activity reached its peak at early stationary phase and then remained continuous during this stage of development for a certain period (approximately 9 hr). The stationary phase of bacterial growth began at 6th hr. A decrease in optical density after 24 hr of incubation was noted at 15th hr from the start of incubation period with a decrease in the activity rate.

Fig. 7 : Surface structure of *E. coli* EMH-542017 cell by Transmission Electron Microscope: (A) normal cells, (B) and (C) after treated with cinnamon and clove oils.

These findings of this demonstrated that essential oils exhibit antibacterial activities, substantially affecting the growth rate of new strain EMH-542017, and the concentration of essential oil influences the antibacterial effects on pathogenic bacteria. Concentrations of 12.5, 25, and 50 $\mu\text{l ml}^{-1}$ of the oil completely eliminated the initial inoculum of *E. coli* after 3 h. The cytotoxic effects of essential oils are mainly caused by their disruption of the cell membrane structure, leading to the permeability of bacterial cells. Cloves also have some medicinal properties, such as anti-inflammatory and antioxidant properties (Fig. 6).

The changes in cells observed in cells treated with cinnamon and clove oils for 24 hr are shown in Fig. 7. This confirmed the potential role of cinnamon bark oil and its major component (cinnamaldehyde) in enhancing the activity of some antibiotics against ESBL-producing *E. coli* isolates. Observations on the abilities of essential oils to induce changes in cell walls and internal inclusions are clearly shown in Fig. 7, in which flagella are absent or lost after *E. coli* were exposed to essential oils. *E. coli* normally possess peritrichous flagella and are known for their swarming ability. Flagella-mediated motility, both swimming and swarming, is an important virulence factor for pathogenic bacteria, which enables them to and during a UTI and prevents *P. mirabilis* from traversing from one kidney to the other via the ureters. Meanwhile, the cell wall seemed to be affected or damaged (Fig 7); the cell wall had played a vital role in the secretion of urease enzyme, which hydrolyzes urea into ammonia and carbon dioxide, resulting in an increase in pH, forming urinary stones and causing inflammation (Hassan et al., 2018).

Clove oil because of its antimicrobial characteristics is used for pathogenic bacterial control (Packyanathan and Prakasam, 2017). Essential oils can be used as alternatives to treat many infectious diseases (Packyanathan and Prakasam,

2017; Parvez and Rahman, 2018; Hassan et al., 2018). Clove oil has antimicrobial, antifungal, antiseptic, antiviral, stimulant, and aphrodisiac properties, in addition to different basic minerals and vitamins, especially vitamin A, and may offer health advantages. Clove oil is used in dentistry and is a significant component of eugenol, which is used to decrease nerve pain (Abdel-Moaty et al., 2016).

Based on our results, we conclude that the new strain *E. coli* EMH-542017 is an ESBL producer. *E. coli* EMH-542017 has multiple virulence factors, broad spectrum resistance against several antibiotics, the ability to produce siderophores, and possesses the ESBL blaCTX-M-9 gene. Essential oils and their major components represent safe, natural, and convenient antibacterial agents against ESBL-producing *E. coli*. Essential oils have great potential to affect local UTI and to prevent bacterial infections.

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Authors' contribution: M.S. Abdel-Hamid, M.M. Hazza: Laboratory work and writing the draft of the manuscript, H.A. Mousa, M. Abd El-Mongy: Writing and editing manuscript, M.F. Awad: Funded this work.

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