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In Vitro Antibacterial Activity of Sweet Basil Against*Escherichia coli* O157:H7 Analyzed Using TEM

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Abstract

Escherichia coli O157: H7 is a known pathogen responsible for several outbreaks and cases of life-threatening hemorrhagic colitis and hemolytic uremic syndrome. Based on our previous results reported on the activity of basil oil against the pathogen, the oil was bacteriostatic and bactericidal at 0.25 and 0.5 mg/ml, respectively. Therefore, these two concentrations were used in this study to observe their effects on the pathogen under a transmission electron microscope. Stationary phase *E. coli* O157: H7 cells were treated with the oil. Untreated *E. coli* O157: H7 was used as a control. A standard procedure for preparing electron microscopy samples was followed.Transmission electron microscope (TEM) images showed that *E. coli* O157: H7 control cells were rod-shaped and had cellular integrity. The treated cells showed clear ultrastructural damage. When the cells were treated with the 0.25 mg/ml oil, the cell membrane was separated from the cell wall and the cell size was reduced. In addition, irregular shape of the treated cells was also detected. During this time, significant damage was found in cells treated with 0.5 mg/ml oil when cell integrity was completely lost and leakage of cell contents was also observed.

Keywords:TEM, *E. coli* O 157:H7, Medicinal plants, Basil oil.

1. Introduction

E. coli O157: H7 has become one of the most important pathogens in food and water. There is a well-known pathogenic association with several epidemics and cases of life-threatening haemorrhagic colitis and hemolytic uremic syndrome, especially in children. The ability of *E. coli* O157: H7 strains to cause serious disease in humans is related to their ability to secrete Shiga toxins. Two main groups of Shiga toxins are known, Stx1 and Stx2**(Smith *et al.*, 2013)**.

Due to their availability and fewer side effects (compared to available antibiotics), the belief that plant extracts are a possible source of antibiotics could be a successful benefit in the treatment of microbial diseases**(Hyldgaard*et al.*, 2012; Bouzidi *et al.*, 2016)**.

Essential oils are fragrant metabolites found in the leaves, roots, bark, fruits and seeds of aromatic plants**(Nazzaro*et al.*, 2013; Swamy*et al.*, 2016)**. It has a long history of use in food processing,

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cosmetics and in traditional medicine. A large number of pharmacological studies related to bactericidal, antifungal, antiviral, antioxidant, anti-inflammatory, chemotherapy and insecticidal activity of essential oils have already been reported**(Rothan*et al.*, 2014; Lakehal*et al.*, 2016)**. Due to the potent pharmacological properties mentioned above, essential oils play an important role in the fight against pathogenic bacteria**(Rothan*et al.*, 2014; Lakehal*et al.*, 2016; Swamy*et al.*, 2016)**. The main active ingredients of essential oils are phenols, terpenes and aldehydes, which are mainly responsible for biological activity**(Reichling*et al.*, 2009)**.

Sweet basil(*Ocimumbasilicum* L., family Lamiaceace) is one of the most popular aromatic plants grown worldwide. It is often called Sweet Basil and in Iraq is called Rehan. The plant has long been used to treat headaches, coughs, respiratory infections, kidney failure and toxins. Leaves, stems and flower parts of the plant are used in traditional medicine as an antiseptic, preservative, sedative, digestive system and a diuretic. In addition, its essential oil has been used as a natural additive to food and has been shown to be active against various types of pathogenic bacteria and fungi. The main constituents of basil oil are monoterpene and phenylpropanoid derivatives**(Lee *et al.*, 2005; Hussin*et al.*, 2008; Poonkodi, 2016)**.

Preliminary data from our previous studies using a time elimination assay and scanning electron microscopy highlighted the antibacterial activity of basil essential oil against *E. coli* O157: H7**(Yakob, 2015; 2016)**. Therefore, this study would be the first study in Iraq to investigate the possible mechanism of basil oil in an important pathogenic *E. coli* strain analyzed at the ultramorphological level using transmission electron microscopy.

2. Materials and Methods

2.1 Isolation of Plant essential oil

The whole plant of *O. Basilicum* was collected at the flowering stage. The plant was identified by Prof. Dr. Ali Fadam Al-Mohammadi from the Center of Desert Studies, University of Anbar. After the leaves of the plant have been separated and washed, the essential oil was extracted by water distribution using a Clevenger device. The collected oil was stored in a dark glass bottle at 4 ° C before use.

2.2 Preparation of *E. coli* O157:H7 cells for TEM

Based on our previous results reported on the concentration-dependent activity of basil herb oil against *E. coli* O157: H7, the oil was 0.25 and 0.5 mg/mlbacteriostatic and bactericidal, respectively**(Yakob, 2015; 2016)**. Therefore, these two concentrations were used to study the effect of the oil on the ultrastructure of the pathogen under TEM.

Stationary phase *E. coli* O157: H7 cells were exposed to the oil at the required concentrations at 37 ° C for 8 hours. The control group of bacteria was treated with only 10% DMSO. The tested cells were then centrifuged at 5000 xg for 5 minutes and washed twice in phosphate buffered saline. The microbial granules, which were not treated or treated with the oil, were first fixed for 2 hours at room temperature in McDowell-Trump fixator (consisting of 50 ml 0.1 mol/L phosphate buffer, 11 ml 37% formaldehyde , 4 ml 25% glutaraldehyde and distilled water to 100 ml). The samples were then rinsed three times

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with 0. 1 mol/L phosphate buffer (pH 7. 2). The granules were fixed in 1% osmium tetroxide in phosphate buffer for 1 hour at room temperature. The samples were then centrifuged and the granules resuspended with distilled water. This step was repeated twice. A drop of 2% agar was added to the solid granule and immediately poured onto a glass microscope disk. After 1 to 2 minutes, the solidified agar containing the cell masses was cut into 1 mm 3 blocks. Thereafter, the samples were dehydrated by consecutive weeks in 50% and 75% ethanol for 15 minutes each, two weeks in 95% ethanol for 15 minutes, two weeks in 100% ethanol for 30 minutes each, and finally two weeks in 100% acetone for 10 minutes each. Cell blocks were infiltrated with acetone and Spurr resin mixture (1:1 v/v) for 15-30 minutes, then with Spurr resin mixture overnight alone, and finally by mixing Spurr's resin at 37 ° C for 5 hours. All the infiltration steps were performed in a rotator at room temperature. After infiltration, bacterial blocks were incorporated into Araldite resin using plastic molds and cured at 60 ° C overnight. Small blocks of bacteria were removed from the plastic molds and cut by Ultramicrotome (Sorvall® Ultra Microtome MT500, USA). Then, ultra-thin sections (90 nm) were stained with 2% (v/v) uranium acetate and Reynold lead citrate, sequentially. After drying, the samples were mounted on 400 mesh copper gratings and then observed with a transmission electron microscope (TEM, LIBRA, Carl-Ziess SMT,Oberkochen, Germany) operating at a voltage of 80 kV.

3. Results and Discussion

Sweet basil has been used in traditional medicine all over the world since the earliest times. The oil has a wide variety of therapeutic uses, including antibacterial activity. Recently, much attention has been paid to basil oil in food preservation, as it can be used as a natural food additive to control the growth of food-borne pathogenic microbes **(Politeo*et al.*, 2007; Swamy and Sinniah, 2015)**. Many researchers in different parts of the world isolated basil essential oil and reported its antimicrobial activity. In this ongoing project, we investigated the possible mechanism of action of basil oil on the ultrastructure of a major pathogen, *E. coli* O157: H7, using transmission electron microscopy.

In Images 1, 2 and 3 transmission electron microscope photographs of *E. coli* O157: H7 cells untreated (control) and treated with basil oil, respectively.

Representative images of untreated bacterial cells showed normal structure for rod-shaped cells. The bacterial cell had cytoplasmic membrane integrity with periplasmic space and the outer membrane was attached to the plasma membrane. The intracellular content is very homogeneous with grains dense in normal electrons (Image 1).

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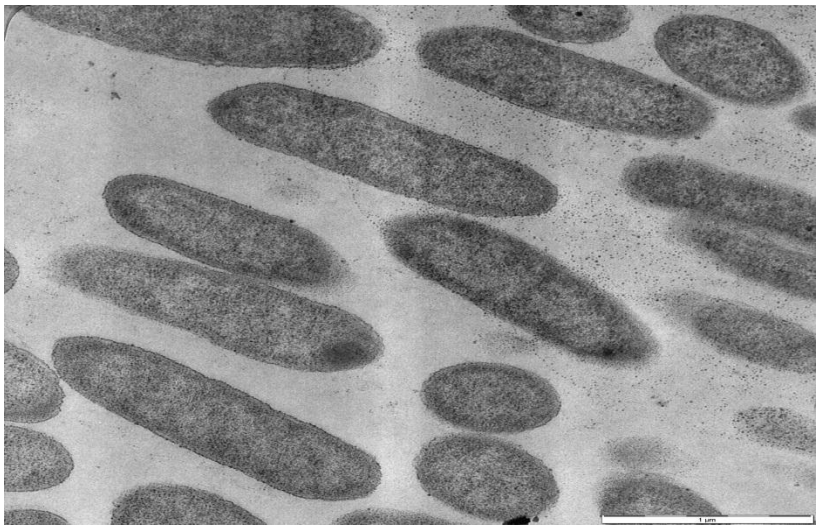

Image 1: Transmission electron microscopy micrograph of untreated *E. coli* O157:H7 cells.

In contrast, significant ultrastructural changes occurred in the examination of *E. coli* O157: H7 cells after exposure to the oil at the two concentrations tested. When the cells were treated with the lowest concentration of basil oil (0. 25 mg/ml), the cell membrane was separated from the cell wall and the cell size was reduced. In addition, an irregular shape of the treated cells was also detected (Image 2).

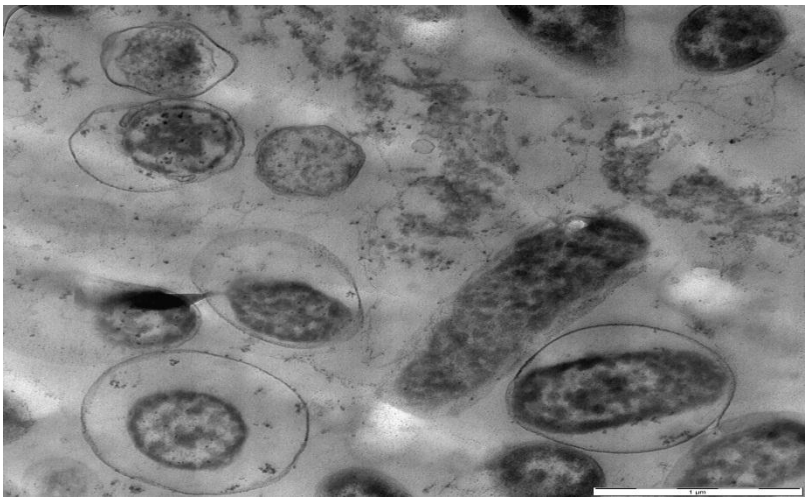

Image 2:Transmission electron microscopy micrograph showing the ultrastructural changes of *E. coli* O157:H7 cells treated with 0.25 mg/ml basil essential oil.

Although irreversible damage was shown when cells were exposed to 0.5 mg/ml oil. The integrity of the cells was lost. Disruption occurred in the outer membrane and cytoplasm of the membrane. Furthermore, cellular leakage was observed in many cells, as they appeared empty (Image 3). These observations indicate the bactericidal effect of the oil at this concentration against *E. coli* O157: H7. It can be suggested that the oil, in the initial step, binds to the bacterial cell surface and penetrates the cell wall, causing damage to the plasma membrane and leading to cell death.

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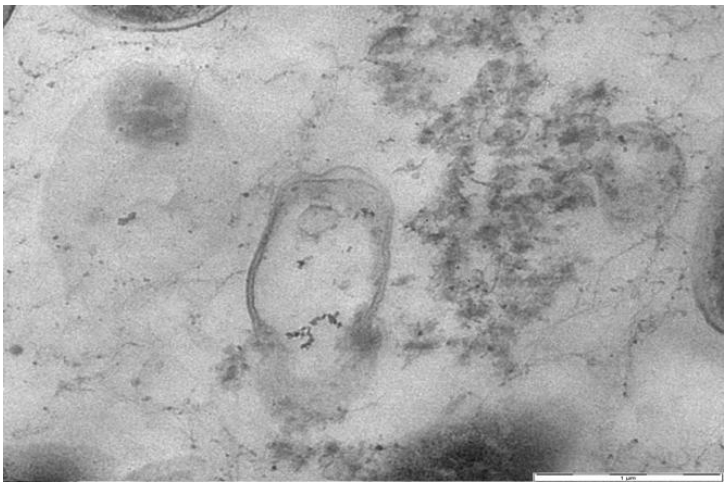

Image 3: Transmission electron microscopy micrograph showing theultrastructural changes of *E. coli* O157:H7 cells treated with 0.5 mg/ml basil essential oil.

The results obtained are comparable to previous studies in which it has been reported that essential oils, due to their hydrophobicity, can interact with the bacterial phospholipid bilayer and disrupt the structural organization of the bacterial membrane, leading directly to the loss of intracellular materials **(Ultee*et al.*, 1999; 2002;Ultee*et al.*, 2002; Fitzgerald *et al.*, 2004; Gill and Holley, 2006; Liang *et al.*, 2011)**. Data from the available literature show that the main chemical constituents of essential oils of the Ocimum species are monoterpene derivatives (especially linalool, camphor, limonene, 1,8-cineol and geraniol) and phenylpropanoid derivatives (especially eugenol, methylugenol,chavicol, Estragole and methyl Cinnamate) **(Lee *et al.*, 2005; Hussin*et al.*, 2008; Bassolé*et al.*, 2010;Poonkodi, 2016)**. Antibacterial properties of these components against several pathogens have been reported **(Pascual Villalobos and Ballesta Acosta, 2003; Nerio*et al.*, 2010)**.

These phenolic active ingredients, especially eugenol and linalool, can interact with enzymes located on the cell wall and attack phospholipids present in cell membranes, which leads to increased permeability Loss of cytoplasmic material **(Ultee*et al.*, 2000; Gallucci*et al.*, 2009;Bassolé*et al.*, 2010; Rattanachaikunsopon and Phumkhachorn, 2010)**.

Conclusions

Based on current results, TEM observations confirmed the antibacterial efficacy of basil essential oil against an important bacterial pathogen, *E. coli* O 157: H7. The findings suggest that the oil's main sites of action appear to be the cell wall and cytoplasmic membrane.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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