

Mechanism of Antimicrobial Action of *Ocimum Basilicum* Essential Oil Against Nosocomial Bacteria

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Abstract: Nosocomial infection is a major concern in the healthcare sector. *Ocimum basilicum* essential oil is one of the main essential oil being investigated for its antimicrobial activities. This study was conducted to determine the effect of *Ocimum basilicum* essential oil on bacterial cell growth and cell membrane integrity. **METHOD:** The *O. basilicum* essential oil (EO) was purchased from Natur Aromatherapy and Wellness and different concentrations of the essential oil (0.092 – 1.470 g/ml) was prepared using 10% DMSO. Disc diffusion test and MIC using broth dilution for *Escherichia coli* and *Staphylococcus aureus*, with Gentamicin as positive control were conducted. 24-hour growth kinetic analysis of the bacteria and their cell membrane integrity were investigated by measuring the absorbance value. **RESULT:** The zone of inhibition by the essential oil was greater in *S.aureus* compared to *E.coli* and the MIC was 0.368g/ml and 0.735g/ml respectively. EO concentrations at 1.47 g/ml and 0.735 g/ml are the only one showed effective inhibition on the growth of the bacteria. The EO was more effective in inhibiting the growth of *S.aureus* than *E.coli*. The absorbance of the bacterial cell constituents increased from the negative control, 2 x MIC, MIC and Gentamicin in both bacteria. **CONCLUSION:** *Ocimum basilicum* essential oil exert its antimicrobial action by affecting bacterial growth and acting on cell membrane integrity of the bacteria.

1 INTRODUCTION

Nosocomial infections are infections which are acquired by patients under medical care during their hospital stay and also by the visitors and healthcare workers. It is also known as hospital acquired infection (Khan *et al.*, 2015). The International Nosocomial Infection Control Consortium (INICC) has reported the rates of device-associated hospital acquired infections (DA-HAIs) in various countries including Malaysia, from 2007 to 2012 is high (Rosenthal *et al.*, 2015). The agents most commonly associated with nosocomial infections are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Streptococcus spp.*, *Acinetobacter spp.*, *Bacillus cereus*, *Proteus mirabilis* and *Serratia marcescens* (Khan *et al.*, 2015).

Essential oils or also known as volatile oils are the mixture of volatile, organic compounds that comes from one plant source and it is the essence that is responsible for the flavor and fragrance of the plant. They can be obtained from different parts of

plants (Tisserand & Young, 2014). They are secondary metabolites of plants which are produced in response to stress. There are three broad categories of plant secondary metabolite that encompass terpenes and terpenoids (oxygenated compound), alkaloids and the phenolic compounds (Hamid *et al.*, 2014). Apart from that, alcohols, esters, aldehydes and ketones can also be discovered on essential oil (Worwood, 1991).

Ocimum basilicum is an aromatic herb with a strong odour and sharp taste that belongs to the Lamiaceae family which has white, rose or violet labiate flowers with bilabiate calyx and four lobed corolla (Moghadda *et al.*, 2011). The plant is used as traditional medicine for gastrointestinal problems like diarrhea, dyspepsia, intestinal spasm and gastritis, also as antitussives, antihelminths and analgesics (Shirazi *et al.*, 2014). The main constituents of *O.basilicum* essential oil are chavicol methyl ether or astragole, linalool and eugenol where the antibacterial activity is said to be due to linalool (Moghadda *et al.*, 2011). It displayed significant antibacterial activity against *Staphylococcus aureus*, *Salmonella enteritidis* and

Escherichia coli while being antiseptic against *Proteus vulgaris*, *Bacillus subtilis* and *Salmonella paratypha* (Shirazi *et al.*, 2014).

2 MATERIAL & METHODS

2.1 *Ocimum basilicum* Essential Oil Acquisition

The *Ocimum basilicum* essential oil was purchased at Natur Aromatherapy and Wellness.

2.2 Antimicrobial Susceptibility Test by Disk Diffusion Method

An inoculum suspension was prepared by inoculating some of the colony from the bacteria subculture into a sterile saline solution. This inoculum suspension was used within 15 minutes to avoid further bacteria growth. The bacteria inoculum was inoculated on Mueller Hinton agar plate. On each plate, the *Ocimum basilicum* essential oil disk (0.092 g/ml) will be placed. The positive control is gentamicin disc (10 µg). The agar plate was incubated at 37 °C for 24 hours. After that, the zone of inhibition of each agar was measured and sensitivity of the bacteria to the essential oil was identified. This experiment was conducted in three replicates (Luis *et al.*, 2016).

2.3 Minimum Inhibitory Concentration using Broth Dilution Method

1 ml of peptone water was placed inside microcentrifuge tube, then 100 µl of bacterial inoculum was placed inside each test tube. 100 µl of *Ocimum basilicum* essential oils which has been diluted by two fold dilution, was placed in to each respective microcentrifuge tube. The mixtures are mixed well then incubated for 24 hours at 37 °C. The control consists of 100 µl gentamicin solution. The lowest concentration of the essential oil that causes complete inhibition of the bacterial growth will be taken as the MIC. This experiment was conducted in three replicates (Luis *et al.*, 2016).

2.4 Growth Kinetic Analysis of *Staphylococcus aureus* and *Escherichia coli* Bacteria

0.3 ml bacterial inoculum suspension was placed into a test tube containing 8 ml of peptone water and they were mixed well by slightly vortexing them. The mixture was then placed into the test tube which contained 0.092 g/ml *Ocimum basilicum* essential oil. The process was repeated for each concentrations of serially diluted *O. basilicum* essential oil. The positive and negative controls in the experiment were prepared by using 0.3 ml gentamicin solution and 0.3 ml peptone water respectively. The test tubes were incubated at 37 °C and analyzed over period of 24 hours (Theophel *et al.*, 2014). A sample from each test tubes were taken at different time intervals and analyzed at 660 nm using the visible spectrophotometer. The absorbance value was recorded and the figure 1 of Absorbance against Time was plotted. The experiment was carried out in three replicates.

2.5 *Staphylococcus aureus* and *Escherichia coli* Bacterial Cell Membrane Integrity

100 ml of bacterial inoculum suspension was prepared and centrifuged for 12 minutes at 3000g. The solution was drained from the centrifuge tube and bacterial cells which was suspended at bottom of tube was retained. The bacterial cells were washed for three times and resuspended using 0.1 M phosphate buffer solution (PBS, pH 7.4). The cell suspension (100 mL) was incubated at 37 °C under agitation for 4 hours in the presence of two different concentrations of *Ocimum basilicum* essential oil (MIC, and 2 x MIC). Then, 25 ml sample from each centrifuge tubes were collected and then centrifuged at 6000 g for 5 minutes (Zhang *et al.*, 2015). The supernatant (3 mL) from each samples were taken and their absorbance value was measured using UV spectrophotometer at 260 nm wavelength. Same procedure was conducted for positive and negative control. (Diao *et al.*, 2014). This experiment was carried out in three replicates.

3 RESULTS

3.1 Antimicrobial Susceptibility Test by Disk Diffusion Method

Disk diffusion method was conducted by Kirby-Bauer method. The essential oil was compared to gentamicin in terms of the zone of inhibition and it showed smaller diameter of inhibition (Table 1).

Table 1 The mean diameter zone of inhibition of *Ocimum basilicum* essential oil against *Escherichia coli* and *Staphylococcus aureus*

Extract	Mean zone of inhibition (cm) ^a	
	<i>E. coli</i>	<i>S. aureus</i>
<i>O. basilicum</i> EO (0.092 g/ml)	0.700 (±0.173)	0.733 (±0.252)
Gentamicin (10 µg)	3.000 (±0.200)	3.467 (±0.153)

^aValues represent three independent replicates ±SD with significant differences between the groups (p<0.05).

3.2 Minimum Inhibitory Concentration using Broth Dilution Method

Broth dilution method were used in minimum inhibitory concentration test where

peptone water was mixed with the essential oil and then was serially diluted by two folds. Table 3.2 showed that the concentration of essential oil needed to inhibit the *Escherichia coli* (*E.coli*) was higher.

Table 2. The minimum inhibitory concentration of *Ocimum basilicum* essential oil against *Escherichia coli* and *Staphylococcus aureus*

	Minimum Inhibition Concentration (MIC) (g/ml)
<i>E.coli</i>	0.735
<i>S. aureus</i>	0.368

3.3 Growth Kinetic Analysis of *Staphylococcus aureus* and *Escherichia coli* Bacteria

Both of the bacteria's growth kinetic were analyzed for a period of 24 hours upon 4 hours treatment with *Ocimum basilicum* essential oil at different

concentrations. From figure 1 and 2, the negative control which consists of bacteria in peptone water only displayed the growth curve and act as the main comparison to the bacteria growth when treated with the essential oil. *E.coli* showed less susceptibility to the essential oil in comparison with *S. aureus*.

Figure 1 Growth kinetic of *Escherichia coli* after treatment with *Ocimum basilicum* essential oil

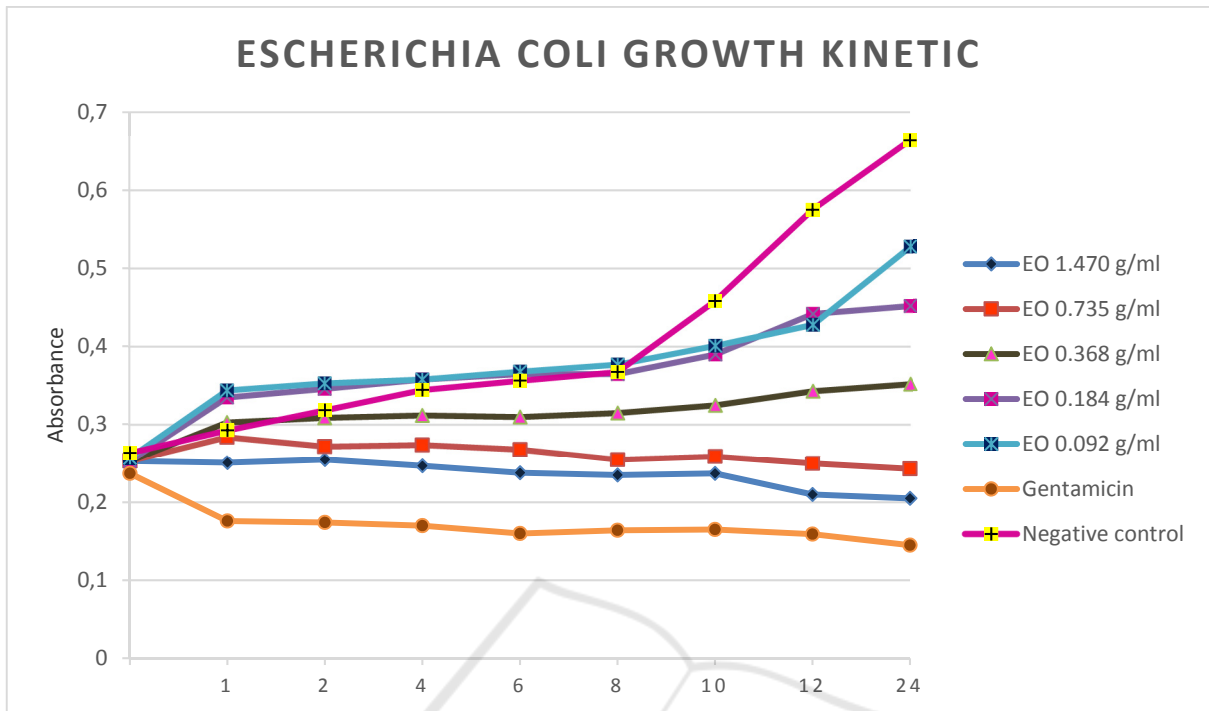
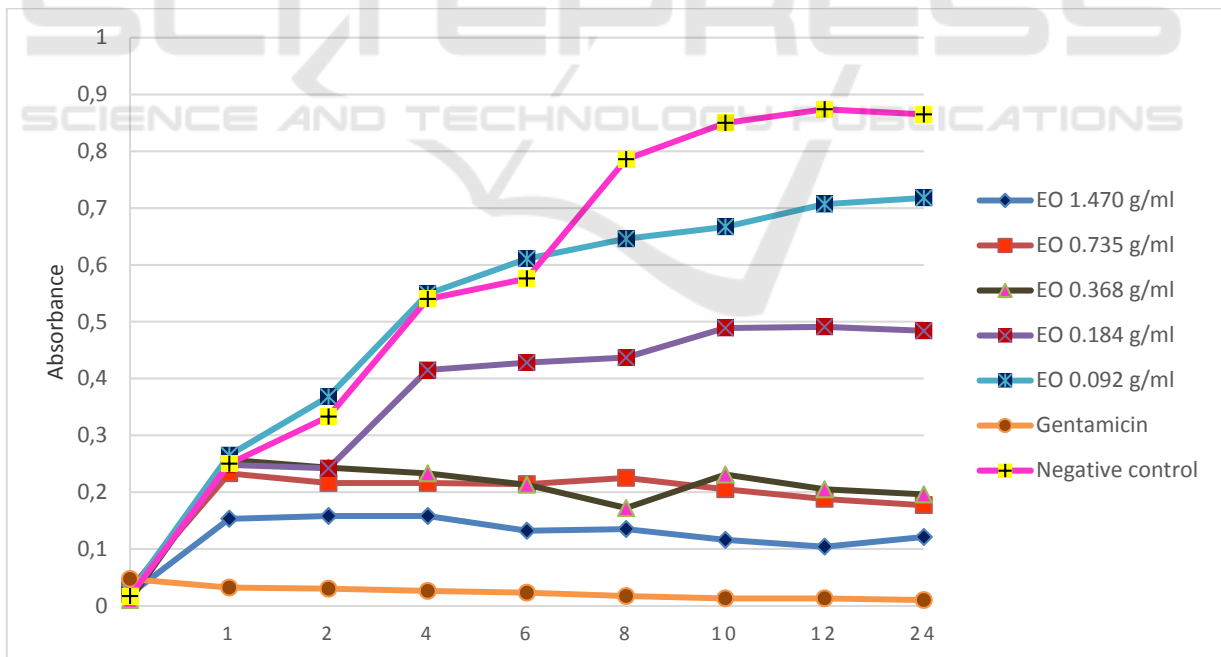


Figure 2 Growth kinetic of *Staphylococcus aureus* after treatment with *Ocimum basilicum* essential oil



4 DISCUSSIONS

Ocimum basilicum (*O. basilicum*) essential oil is known for its antibacterial activity and this properties can be used in reducing the nosocomial infections in healthcare setting. The result from the study showed that *O.basilicum* essential oil has statistically significant effect on the growth and cell membrane integrity of bacteria. *O. basilicum* essential oil was tested for antimicrobial susceptibility and the outcome was reflected by the presence of zone of inhibition (ZOI) in the disk diffusion test. It was observed that the zone of inhibition was larger in *Staphylococcus aureus* (*S. aureus*) compared to *Escherichia coli* (*E. coli*). However, the difference in diameter zone of inhibition between the two bacteria was small where ZOI of *E.coli* was 0.700 cm and *S. aureus* was 0.733 cm. Both of the bacteria were susceptible to Gentamicin antibiotic (positive control). Gentamicin also showed larger inhibitory zone in *E. coli* than *S. aureus* with 3.000 cm and 3.467 cm respectively.

The minimum inhibitory concentration was determined for both bacteria by broth dilution method. In this method, two fold dilution of *O. basilicum* essential oil was done to prepare series of concentration of 1.470 g/ml, 0.735 g/ml, 0.368 g/ml, 0.184 g/ml and 0.092 g/ml. After an overnight incubation, results showed that the MIC for *E.coli* and *S. aureus* were 0.735 g/ml and 0.368 g/ml respectively. The lowest concentration of *O. basilicum* essential oil that was able to inhibit the growth of *E. coli* was higher than *S. aureus*.

From the antimicrobial susceptibility study that was conducted, *O.basilicum* essential oil showed more antimicrobial activity against gram positive bacteria than gram negative bacteria. In the study done by (Shirazi *et al.*, 2014). *O.basilicum* essential oil also showed stronger effect on gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* compared to the gram negatives *Escherichia coli* and *Salmonella typhi*. The presence of outer membrane which consists of lipopolysaccharides enables the gram negative bacteria to protect itself by restricting the penetration of hydrophobic compounds like the essential oil. Thus, the essential oil may not be able to properly attack the phospholipid layers of bacteria cell to compromise its permeability and integrity. *O.basilicum* essential oil exhibited high antibacterial activity which is associated with its major constituents of astragole and linalool (Moghadda *et al.*, 2011; Shirazi *et al.*, 2014).

Based on the figure 1 and 2, *Ocimum basilicum* (*O. basilicum*) essential oil effectively inhibits the growth of *Staphylococcus aureus* (*S. aureus*) more than that of *Escherichia coli* (*E. coli*). In the growth kinetic analysis of *E. coli*, *O. basilicum* essential oil was able to effectively inhibit the growth of bacteria with the concentration of 1.470 g/ml and 0.735 g/ml which was represented by the steady decline of the line graph. Starting at concentration of 0.368 g/ml and lower, the line graph started to increase steadily. At concentration 0.368 g/ml of the essential oil, inhibitory action on the bacteria growth occurred up to time 6 hours only, then the bacteria growth curve began to increase. At concentration of 0.184 g/ml and 0.092 g/ml of the essential oil, it was observed that the inhibitory action is not effective as the bacteria continued to grow steadily at lower rate compared to that of the negative control (containing only peptone water and *E. coli* bacteria). In the positive control test, it was showed that it is able to significantly decrease the growth of *E. coli* and the line graph decreased and remained at a low growth rate throughout the 24 hours study. In *S. aureus* growth kinetic analysis, the growth of bacteria was inhibited at concentration of 1.470 g/ml, 0.735 g/ml and 0.368 g/ml. It was observed that at concentration of 0.184 g/ml and 0.092 g/ml, the growth of *S. aureus* continued and compared to the other concentrations of the essential oil the difference is large. However, this growth was at lower rate if compared to the negative control. Gentamicin was also effective in inhibiting *S. aureus* growth.

From the growth kinetic analysis study, it was found that *O.basilicum* essential oil effectively inhibit bacteria growth at higher concentrations. Meanwhile, gentamicin was able to inhibit the bacteria growth more than that of *O.basilicum* essential oil. In the plotted line graph based on the study, the normal bacteria growth phase which consists of the lag phase, log (exponential phase), stationary phase and death phase were not clearly defined as the study was done only for a 24 hour period. Nevertheless, from the study we managed to confirm that *O.basilicum* essential oil was able to inhibit bacteria growth. *O.basilicum* essential oil acts by different pathways or mechanism in which it exerts its antibacterial activity such as through compromising bacteria membrane permeability and integrity, inhibiting ATP production, coagulation of bacteria cell constituents and inhibiting bacteria quorum sensing (QS) system (Aiemsaard *et al.*, 2014; Luis *et al.*, 2016). The essential oil of

O. basilicum plant may inhibit the bacteria growth through one or more of these mechanisms.

There are various mechanisms of antimicrobial action for essential oil that have been proposed; however, each essential oil has different mechanisms and it is crucial to investigate the exact mechanism to develop a more specific antimicrobial agent. *Ocimum basilicum* (*O. basilicum*) essential oil has the characteristic of being lipophilic and it is able to penetrate the cell membrane of bacteria and accumulate there, which then disrupts its integrity, causing release of cell constituents such as proteins, nucleic acids, and reducing sugars (Diao *et al.*, 2014).

This is confirmed by the result of the study where there is an increase in the absorbance value of the sample when the concentration of *O. basilicum* essential oil was increased from MIC to 2x MIC. The absorbance value increased from 3.811 to 4.053 when the concentration of essential oil was increased from MIC to 2x MIC in *E. coli*, which is also displayed in *S. aureus* (from 4.077 to 4.494). It was observed that the effect of *O. basilicum* essential oil in compromising the cell integrity was more prominent in *S. aureus* than *E. coli*. Gentamicin was also able to disrupt bacteria membrane integrity and from this study, it gave a higher absorbance value than *O. basilicum* essential oil. From the study, it was confirmed that *O. basilicum* essential oil was able to compromise bacteria cell membrane integrity. Cell constituents such as proteins and nucleic acid are important in maintaining cell structural integrity and cell genetic identification respectively. When the cell loses such important constituents, the cell normal functioning in DNA transcription and translation is compromised and this eventually leads to cell death (Zhang *et al.*, 2015).

In the current study, effects of *Ocimum basilicum* (*O. basilicum*) essential oil were studied in *Escherichia coli* and *Staphylococcus aureus*. It will be more beneficial if other gram negative and gram positive bacteria and fungal which are commonly associated with nosocomial infection were studied. A study on which method of extraction for *O. basilicum* essential oil may be carried out which can help researchers to determine which method produced higher yield. Moreover, other tests such as cell membrane permeability and scanning electron microscope (SEM) can be conducted to further investigate how *O. basilicum* essential oil alters bacterial cell membrane permeability, which allows leakage of electrolytes and also how it causes changes in the bacteria morphology.

5 CONCLUSION

Based on the results, it was concluded that *Ocimum basilicum* essential oil exerts its antimicrobial action against bacteria by inhibiting their growth and disrupting the cell membrane integrity. The statistical analysis using SPSS version 21 indicated that there was a significant difference in the growth and cell membrane integrity of *Escherichia coli* and *Staphylococcus aureus* upon treatment with *Ocimum basilicum* essential oil. The essential oil at higher concentration was effective. The essential oil's ability to disrupt the bacteria cell membrane integrity causes leakage of cell constituents and ultimately leads to cell death. Thus, *O. basilicum* essential oil can be used as a natural alternative agent for the treatment of nosocomial infection.

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Conflict of Interests

Author declares there is no conflict of interest.

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