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Antimicrobial Activity of Combined Cinnamon Nano Emulsions-Antibiotics against *Acinetobacter Baumannii*

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ABSTRACT

Threats of *Acinetobacter baumannii*, a Gram-negative and multi-drug resistant bacterium, to the public health have recently received great attention worldwide. This is because the high incidence rate of the bacterial infections and the ability of the *A. baumannii* to resist a wide range of antibiotics. This study is designed to investigate the antimicrobial activity of the cinnamon oil in water as Nano emulsions (NEs) alone and in combination with different antibiotics against clinical isolate of *A. baumannii*. At the beginning, the minimum inhibitory concentration (MIC) of different antibiotics and also cinnamon Nano emulsions (CNEs) are determined. Later, the synergistic effect of the CNEs with each of the studied antibiotics is also examined. Results showed that the bacterium has the highest resistant towards ampicillin (MIC = 700 µg/ml), followed by kanamycin, and gentamycin with MIC of each of the antibiotics was 200µg/ml and 150 µg/ml, respectively. Cinnamon Nano emulsions (CNEs) were also showed a profound inhibitory effect on *A. baummanii*. Interestingly, combinations of cinnamon Nano emulsions (CNEs) with either kanamycin or gentamycin, but not ampicillin, show a significant increase in the antimicrobial activity of each of the antibiotics compared to when they were used alone. The results of this study strongly suggest the potential application of CNEs in combination with antibiotics to overcome *A. baummanii* infections.

Keywords: Cinnamon nano émulsions, Multi Drug résistance Acinetobacter baumannii, Minimum inhibitoire concentration

1. Introduction

Acinetobacter baumannii is an important multi-drug resistant (MRD), Gram-negative opportunistic pathogen that causes a number of serious health issues, including pneumonia, bloodstream, urinary tract, and wound infections in hospitalized patients [1,2]. Recent reports on *A. baumannii* infections alarmed that this pathogen rapidly increased worldwide and serious concern has been paid to minimize the incidence of this pathogen [3]. There are also several reports on isolating of *A. baumannii* among those casualties who were hospitalized in Iraq during Iraqi freedom operation [4-6]. For this reason researchers named this pathogen as "Iraqi bacter" [7,8]. In its annual report of 2017, World Health Organization (WHO) also categorized *A. baumannii* as number one priority pathogen to be tackled through developing new therapeutic agents [9].

Nano emulsions (NEs) are clear emulsified mixtures of particles (average droplet range in size from 50-800 nm) that are made up of water, oil, a surfactant (e.g. Tween 80 and Capriole 90), which is a surface tension reducing agent, and sometimes a co-surfactant (e.g. Propanol and ethanol), which makes a surfactant more effective and enhances its abilities [6,10]. Currently, researchers are mainly focused on using different plant-based oil extracts to prepare NEs due to its bioavailability and biocompatibility [11]. NEs have many applications in the pharmaceutical and food industry; they can work as good delivery systems for both hydrophobic and hydrophilic drugs and also solvents for other non-water soluble drugs [10,12]. In addition, they can easily manufactured and have a stable thermodynamic property [10,12]. As a drug delivery tool, nanoparticles fuse with the lipid-containing plasma membrane of the organism and its potential energy is released from the interactions of the NEs cations and the anions from the cell membrane of the pathogen. This, eventually causes degeneration of the plasma membrane of the pathogen and death [13].

Cinnamon (*Cinnamomum zeylanicum*) has been used for centuries by human as a spice and flavouring agent for cooking and also as medicine (14). The antibacterial activity of cinnamon essential oil exhibited strong antibacterial activities against different clinical isolates of *Escherichia coli* and *Staphylococcus aureus* [15,16]. This study has aimed to investigate the antibacterial activity of CNEs against *A. baummannii* strain H72721 and to determine its synergistic effect, when combined with other antibiotics which are used in the study in hand.

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2. Materials and Methods

2.1 Materials

Tween 80, Tryptic Soy Broth (TSB) and antibiotics were all obtained from Sigma-Aldrich- US. Water was taken from Milli-Q-water purification system (Millipore, MA, USA).

2.2 Bacterial strain

Acinetobacter baumanii strain (H72721) was purchased from American Type Culture Collection (ATCC) (catalog No. NR-9667). The strain was originally isolated in Germany

2.3 Preparation of the Nanoemulsion

Cinnamon Nano emulsions (CNEs) were prepared by mixing locally purchased cinnamon essential oil, deionized water, and 5% tween 80 as a surfactant using high energy method ultrasonicator for 20 minutes until a homogenous suspension was formed. Then, the droplet size was measured by using transmission electron microscopy (TEM) following Gosh *et al.* 2013 (17).

2.4 Determination of minimal inhibitory concentration (MIC)

The antibiotic susceptibility test was carried out as previously described (18). Briefly, the *A. baumanii* strain H72721was grown in 50 ml TSB medium with shaking at 30°C. When OD_{600} reached 0.6, aliquot of the culture was diluted with TSB to OD_{600} ~ 0.1 to be used for antibiotic sensitivity test. Twenty microliters (20 µl) of the diluted culture was mixed with 160 µl of Tryptic Soy Broth (TSB) and 20 µl of the serial dilutions from each of these antibiotics (ampicillin, kanamycin and gentamycin) or CNEs in a 96 polystyrene micro well plates. The concentration ranged from 7 mg to 1 mg of the each of the antibiotic stocks which were used in the experiment followed by10 folds dilution of the antibiotic upon the addition to the well. The micro plate was incubated for 20 hours at 30 °C and the optical density was measured at 600 nm by a micro plate reader.

2.5 Nanoemulsion-Antibiotic synergistic experiment

The synergistic effect of the antibiotics was tested separately with and without adding 0.05% CNEs in a 96-well plate. The bacterial cells were grown in TSB medium as mentioned above. Then, 20 µl of the culture (OD_{600} ~ 0.1) is added to each of the wells containing 160 µl of the medium with 20 µl of 0.05% of CNEs and an antibiotic. The final concentrations of each of the tested antibiotics in the well were 300 µg/ml of ampicillin, 25 µg/ml of kanamycin and gentamycin. The micro plates were incubated for 20 hours at 30°C. Finally, the growth of the bacterial cells was measured by a micro plate reader in the optical density (OD_{600}).

3. Results and Discussion

Massive production, uncontrolled consumption and prescription of antibiotics have led to the emergence of several genera of multi-drug resistant (MDR) bacteria, which are now a serious challenge to the global public health. *A. baumannii* is among the MDR bacteria that the treatment of their infections has become increasingly problematic because of their resistance to multiple classes of antibiotics [3]. Thus, this study is conducted to test the antibacterial activity of cinnamon Nano emulsions (CNEs) and its synergistic action with some antibiotics on a MRD strain of *A. baummannii* H72721. In the beginning, the minimum inhibitory concentrations (MIC) of ampicillin, kanamycin, and gentamycin are determined against *A. baummannii* H72721. The MICs for ampicillin, kanamycin, and gentamycin are found to be 700 µg/ml, 200µg ml^{-1}, and 150 µg ml^{-1} respectively (Figure 1 and 2).

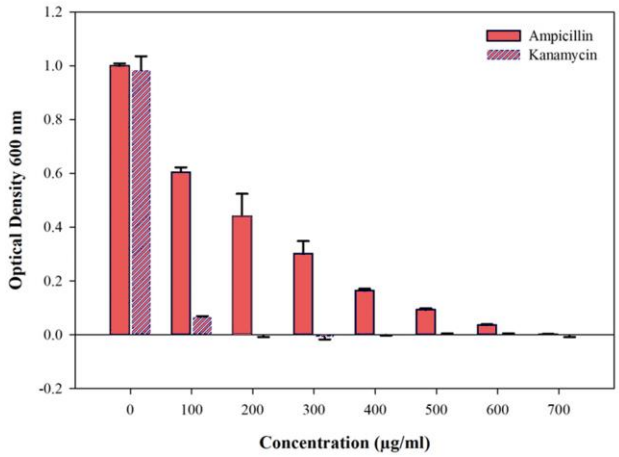

Figure 1: MIC of kanamycin and ampicillin against *A. baumannii*. Growth of *A. baumannii* in TSB 20 hours after incubation at 30 ̊C without any antimicrobial agent and with different concentration of ampicillin and kanamycin. Data shown are the means of data from three replicates ± standard deviations.

The high degree of the bacterial resistance towards ampicillin and less towards kanamycin and gentamycin can be due to the differences in the antibiotic's mode of action. The bactericidal effect of ampicillin occurs as the result of its inhibitory action on the cell wall synthesis of the bacteria [19]. However, kanamycin and gentamycin exhibit antimicrobial activity by inhibiting bacterial translation process through binding to the 16S rRNA component of the 30S RNA [20]. The more consumption and frequent prescription of ampicillin in the clinical settings can also be another factor that bacteria are generally more resistant to ampicillin.

In order to measure the antimicrobial activity of the CNEs, a mixture of cinnamon oil, water and tween 80 was prepared. The average particles size and the percentage of polydispersity of the CNEs were found as 9.35±2.5 nm and 25.73±5 nm, respectively. The antimicrobial property of the CNEs and also their MIC were examined by having 0% (positive control) to 0.15% of NEs in the bacterial culture. The total bacterial growth was inhibited at the present of 0.09% of NEs in the culture (Figure 3).

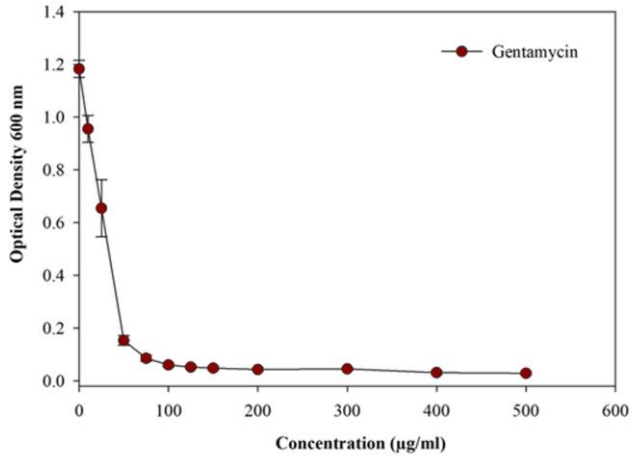

Figure 2: MIC of gentamycin against *A. baumannii.* Growth of *A. baumannii* in TSB 20 hours after incubation at 30 ̊C without any antimicrobial agent and with different concentration of the gentamycin. Data shown are means of data from three replicates ± standard deviations.

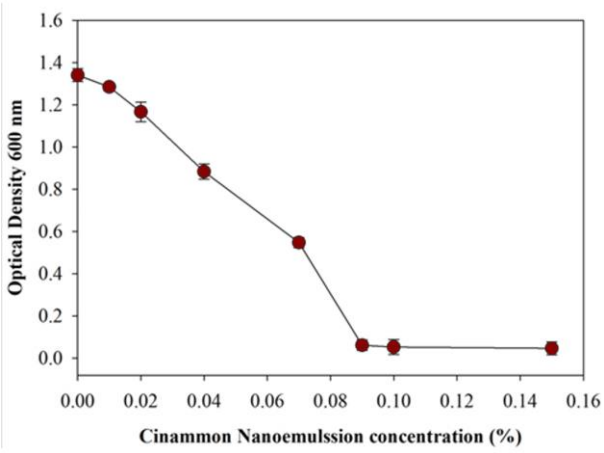

Figure 3: Effect of different concentration of cinnamon nanoemulsion on the growth of *A. baumanni*. Data shown are means of data from three replicates ± standard deviations.

The antibacterial activity of cinnamon essential oil is already reported on different bacterial species [15,16]. In the present study, the antimicrobial activity of the cinnamon essential oil was tremendously increased when it was used in the form of CNEs. This result suggests that *A. baumannii* is very sensitive to cinnamon and this is probably due to the dual actions of both the emulsion and the cinnamon oil constituents on bacterial cells. This can be supported by the ability of NE particles to incorporate into the phospholipid bilayers of the cell membrane. This is followed by destabilizing of the plasma membrane and finally leading to the bacterial cell death [13]. Moreover, the antibacterial activity of cinnamon is ascribed to its bioactive phytochemical constituents such as cinnamaldehyde and eugenol [21]. However, the precise action mechanism of cinnamon constituents on bacterial cells has been left unclear.

Combinations of different antibiotics, such as ampicillin and cloxacillin, are used since a long time to overcome resistant bacteria [22]. The penicillinase inhibitor, clavulanic acid, is mixed with Amoxicillin to treat infections that are caused by penicillinase-producing bacteria [23]. Same approach is taken in this study in order to show the synergistic action of CNEs and the studied antibiotics.

Interestingly, the antimicrobial activity of both kanamycin and gentamycin were significantly increased to 72% and 22% respectively when they were combined with CNEs (Figure 4). However, there was not a significant different in the antimicrobial activity of ampicillin when it was combined with CNEs in the experimental setup (Figure 4).

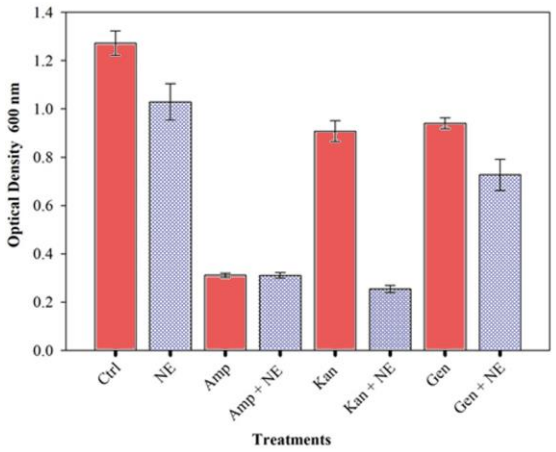

Figure 4: Growth of *A. baumannii* after exposure to 0.04% of cinnamon Nano emulsions alone and with different concentration of different antibiotics. The antibiotics that were used are ampicillin (300 µg/ml), kanamycin (25 µg/ml), and gentamycin (25 µg/ml). Data shown are means of data from three replicates ± standard deviations.

The improvement of the antimicrobial activity of both kanamycin and gentamycin can be due to the dual actions of both CNEs and antibiotics together. In this situation, the simultaneous impairment of the plasma membrane permeability by the CNEs [13] and inhibition of protein synthesis by both the antibiotics occurred in the bacterial cells [20]. The fact of not having an increase in the antibiotic activity of ampicillin when it was combined with CNEs could be due to the ampicillin's mode of action and its bactericidal activity. This means that ampicillin first killed the cells through cell wall disruption and followed by osmolysis [19], so the effect of the CNEs cannot be observed on already dead cells.

4. Conclusion

In conclusion, the results the current study has come across suggest a significant increase in the antibacterial activity of both kanamycin and gentamycin when they are used in combination with CNEs. Thus, such combination could be used to tackle multi-drug resistant *A. baummannii* particularly in topical treatments.

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