

Antibacterial activity of date molasses against *Enterobacter aerogenes* isolated from burns infections in Karbala Province

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ABSTRACT

Background: *Enterobacter aerogenes* is a Gram-negative bacteria belong to Enterobacteriaceae family, widely spread in nature, and it is a part of the normal flora of the intestinal tract. Recently, there have been many reports of increased nosocomial infection with these bacteria, especially in the intensive care unit. *E. aerogenes* opportunistic bacteria mostly causing nosocomial diseases, such as bacteremia, particularly in elderly or debilitated patients, respiratory tract infections, and urinary tract infections, particularly in catheterized patients. **Materials and Methods:** The current study determined the antibacterial activity of date molasses against *E. aerogenes* bacteria which cause burn inflammation of patients who visited or attended Al-Hussein Educational Hospital in Karbala Province. **Results:** The correlation between molasses and antibiotics was evaluated using fractional inhibitory concentration, and the combination results revealed that the antibacterial activity of molasses could be improved by using with antibiotics. The minimum inhibitory concentration (MIC) of molasses reached to 35 mg/ml, but the MIC of molasses in combination with ciprofloxacin and ampicillin reached to 15 and 25 mg/ml, respectively. The ability of *E. aerogenes* bacteria to produce hemolysin toxin, protease enzyme, and biofilm production came to be reduced when treated with MIC of date molasses. The enhanced antibacterial activity of molasses against *E. aerogenes* bacteria when combined with antibiotic opens the way of the other studies to show mechanical work of this combination. **Conclusion:** The main conclusion of this study is the enhanced antibacterial activity of molasses against *E. aerogenes* bacteria when combined with antibiotic, and this opens the way of the other studies to show mechanical work of this combination.

KEY WORDS: *Enterobacter aerogenes*, Minimum inhibitory concentration, Molasses

INTRODUCTION

Enterobacter aerogenes is a Gram-negative bacteria belong to Enterobacteriaceae family, widely spread in nature, and it is a part of the normal flora of the intestinal tract.^[1] Recently, there have been many reports of increased nosocomial infection with these bacteria, especially in the intensive care unit, and there were no records of such infections before 1980.^[2] *E. aerogenes* opportunistic bacteria mostly causing nosocomial diseases, such as bacteremia, particularly in elderly or debilitated patients, respiratory tract infections, and urinary tract infections, particularly in catheterized patients.^[3] The random and repeated use of antibiotic in the treatment of bacterial infection has increased

in the number of multidrug resistance bacterial strain and thus reducing the therapeutic effectiveness of antibiotics. Alternative strategies to confine the spread of virulence bacterial diseases are in demand due to the risk of further resistance development. One of these alternative strategies is used plant extracts. Scientists have long been interested in medical plants for their use as a mean of a treatment for many cases (for their rapid therapeutic effect and the absence of adverse side effects especially in recent years).^[4] One of these medical plants is the date palms, *Phoenix dactylifera*, represent a living mass of nutrients. Dates contain a high nutritional value of human because of having phenolic compound tannins, fast absorption sugars including glucose and fructose, considered the highest fruit's sugar about 77.93–88.02% of the components of the date contain a very large percentage of the iron component (which prevents anemia and significantly strengthens the blood), calcium, vitamins, potassium

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and phosphorus, and a large percentage of fiber.^[2,5] Date molasses helps in the treatment of accelerated heartbeat because it contains copper and magnesium and treat eye problems from infections, pain, allergies, coughs, respiratory diseases, asthma, and stress. It provides heat and warmth the body.^[6,7] The use of date molasses inhibits the accumulation of bacteria and prevents adhesion to one of the proteins on the surface of infected cells, which helps to facilitate the access of antibiotics and chemicals compounds into the tissues. In addition the antioxidant of date molasses usually accelerates of healing wounds and chronic ulcers in human body.^[8] Therefore, this study was carried out to know the inhibitory efficacy of molasses against *E. aerogenes*, which causes burn inflammation and determination of the efficiency of the inhibitory effects of the date dipping in the control of the factors studied virility.

MATERIALS AND METHODS

Bacterial Isolates

Thirteen burn swabs were collected from patients who attended the burn's unit at Al Hussein Hospital in Karbala Province, by using cotton swabs. Then the sample was cultured on Blood and MacConkey agar which was already prepared according to manufactory instructions. After that plates were incubated at 37°C for 24–48 h and monitored growth. The isolated bacteria were already diagnosed biochemically according to methods described by Collee *et al.*,^[9] Baron *et al.*^[10] Finally, the diagnosis of isolates bacteria was confirmed by using Vaitek device and APi-20E system according to instructions manufacturer (bioMerieux).

Ability of Isolated Bacteria to Produce Virulence Factors

The ability of diagnosed bacteria to form biofilm was determined as described by Mathur *et al.*,^[11] AL-Wazni *et al.*^[12] In addition, the ability of isolated bacteria to produce hemolysin and protease enzyme was determined by culture isolates on blood and skim milk agar plates, respectively, as described by Collee *et al.*,^[9] Baron *et al.*^[10]

Determination of the Minimum Inhibitory Concentration (MIC) of Molasses and Antibiotics

Date molasses got ready from the local markets of the Karbala city. The MIC of the date molasses and each type of antibiotic used in study represented by the last concentration with little or no visible growth. The stock solutions and gradual dilution for molasses and antibiotics were prepared as described by AL-Wazni *et al.*^[12] Then, the MIC of antibiotics (Cip and Amp) were mixed with each dilution of molasses in ratio 1:1 to determine the MIC of molasses in the presence of

antibiotic, then the MIC of molasses was mixed with each dilution of antibiotic (Cip and Amp) in ratio 1:1 to determine the MIC of antibiotic in the presence of molasses. Then, the diameter of inhibition zone of MIC of molasses and antibiotic alone and in combination was accounted to determine the antibacterial activity of it by using agar well diffusion methods.^[12]

Effect of Date Molasses on the Bacterial Virulence Factors

All tests to determine virulence factors of isolated bacteria were made after mixing the bacterial suspension with MIC of molasses and as mentioned in Step 2.

Combination Tests of Molasses and Antibiotic

The effect of combination molasses and antibiotics on the antibacterial activity of each one was evaluated by determining the fractional inhibitory concentrations (FIC) as described by Mandal *et al.*^[13] The FIC value was calculated using the following formula:

$$\text{FIC (antibiotic)} = \frac{\text{MIC of antibiotic in combination}}{\text{MIC of antibiotic alone}}$$

$$\text{FIC (extract)} = \frac{\text{MIC of extract in combination}}{\text{MIC of extract alone}}$$

The interactions indices between the antibiotics and the molasses were assessed by using the FIC indices as described by Al-Wazni and Hadi,^[14] which were calculated by using the following formula:

$$\text{FIC index} = \Sigma \text{FIC} = \text{FIC (antibiotic)} + \text{FIC (molasses)}$$

The combinations types were depended on value of FIC indices, so classified on this basis to several types such as synergistic (FIC indices were <1), additive (FIC indices were 1), indifferent (FIC indices were between 1 and 2), and antagonistic (FIC indices were >2).

RESULTS AND DISCUSSION

Determine of the MIC of Date Molasses

The result, as shown in Table 1, showed that the MIC of molasses was 35 mg/ml, whereas MIC of ampicillin and ciprofloxacin was 95 µg/ml and 60 µg/ml, respectively. When used the date molasses in combination with ampicillin and ciprofloxacin, the MIC decreased to 25 µg/ml of ampicillin and 15 µg ml of ciprofloxacin, and these results refer that date molasses is an effective antibacterial compound, especially when used in combination.

The fruits of dates are known to have many categories of biologically active components such as carotenoids include flavonoids, tannins, sterols and polyphenols, in particular phenolic acids isoflavones, and

lignans.^[8] Researchers were found that date fruits have antibacterial activity and antifungal activity *in vitro* and referred that inhibitory substance activity may be steroidal factors when studied the date extract effete on *Candida albicans*.^[15,16] Studies confirmed that antibacterial effect of date extract through damages the cell membranes and induces aggregation of the cytoplasmic contents.^[16] The components that mediate direct antimicrobial effects are unknown. However, both currently identified and unidentified phytochemicals present in dates may be involved in the antimicrobial activity.^[17]

Antibacterial Activity of Date Molasses

The current study confirms that date molasses was active against *E. aerogenes* in comparison to antibiotics ciprofloxacin and ampicillin as a positive control and distilled water as a negative control. The inhibition zone of date molasses alone was 1 cm, whereas the inhibition zones of ampicillin found 2.8 cm and ciprofloxacin 3.5 cm, while the inhibition zone increased to 4 cm when used the date molasses in combination with antibiotics. The antibacterial activity of date molasses against studied bacterial isolates was confirmed when used a combination of antibiotic and date molasses compared with antibiotic alone or date molasses alone shown in Table 2. A study by El Sohaimy *et al.*^[17] revealed that the date palm fruits are a good source of natural polyphenolic compounds with high antioxidant and antibacterial activities. Their finding was that date palm fruit extract has a strong antibacterial activity against *Escherichia coli*, *Salmonella enterica*, and *Bacillus subtilis* (20 ± 0.57 and 16 ± 0.57 mm), (20 ± 0.54 and 14 ± 0.52 mm), and (18 ± 0.32 , 15 ± 0.23 mm), respectively. While, moderate inhibition the effect of date palm extract against *Staphylococcus aureus* and *Enterococcus faecalis* (8 ± 0.48 and 5 ± 0.52 mm) and (5 ± 0.36 and 2 ± 0.57 mm) respectively.

Antivirulence Activity of Date Molasses

E. aerogenes are pathogenic bacteria which possess a number of virulence factors including biofilm formation, different exotoxin, endotoxin, siderophores, and adhesions.^[18] The production of virulence factors by this bacteria was influenced by added MIC of date molasses or antibiotic to the bacterial suspension. The results showed decline in the ability of bacteria to produce hemolysin toxin and protease enzyme after exposure to MIC concentrations compared to control. Figures 1 and 2 illustrate that *E. aerogenes* was a high producer for biofilm formation though the increase in reading the optical density that reached to 2.2 when it was measured for bacterial suspension alone which used as control. The optical density greatly reduced when added the MIC of date molasses and antibiotic (Cip and Amp) together to *E. aerogenes* suspension before reading the optical density that reached to 0.08 in combination of molasses and ciprofloxacin and to 0.03 in combination of molasses and ampicillin, whereas was 0.85 in added of molasses only to bacterial suspension. The results confirm that biofilm formation activity of *E. aerogenes* bacteria was decline after treatment with MIC of different concentrations of date molasses alone and with combination with Cip or Amp antibiotic. Therefore, the date molasses is one of the most important plant extracts that improve or increase the effect of antibiotic against antibiotic-resistant pathogenic bacteria.^[14,19]

Interactions between the Date Molasses and Antibiotics *In vitro*

The results in Table 3 showed that combination between molasses and antibiotics reduces the MICs of antibiotic against multidrug resistance pathogen and found that the effectiveness of antimicrobial agents was improved by combining the antibiotics with date molasses against *E. aerogenes* isolates *in vitro*. The interaction between molasses and Cip was indifferent

Table 1: MIC of dates molasses and antibiotic against *Enterobacter aerogenes*

Type of component	MIC of component alone	MIC of molasses in combination with antibiotic	MIC of antibiotic in combination with molasses
Date molasses	35 mg/ml	-	-
Ampicillin	95 µg/ml	25 mg/ml	80 µg/ml
Ciprofloxacin	60 µg/ml	15 mg/ml	45 µg/ml
D.W	0	0	0

MIC: Minimum inhibitory concentration

Table 2: Antibacterial activity of date molasses against *Enterobacter aerogenes* bacteria

Type of compound	Inhibition zone diameter rate (cm)
Date molasses (mg/ml)	1 cm
Ciprofloxacin MIC (µg/ml)	3.5 cm
Ampicillin MIC (µg/ml)	2.8 cm
MIC of date molasses (mg/ml) and MIC of ciprofloxacin (µg/ml)	4 cm
MIC of date molasses (mg/ml) and MIC of ampicillin (µg/ml)	3.5 cm
DW	0

MIC: Minimum inhibitory concentration

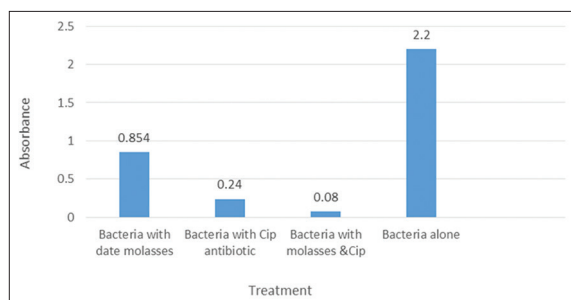


Figure 1: Effect of molasses and ciprofloxacin on biofilm formation of bacteria

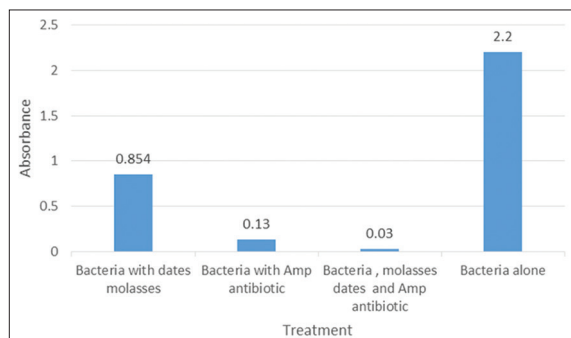


Figure 2: Effect of molasses and ampicillin on biofilm formation of bacteria

Table 3: Effect of the combination between date molasses and antibiotics on *Enterobacter aerogenes* bacteria

Type of antibiotic	Mean FIC of antibiotic	Mean FIC of molasses	FIC index	Interaction
Cip	0.750	0.428	1.178	Indifferent
Amp	0.842	0.714	1.556	Indifferent

against *E. aerogenes* bacteria though its effects to highly reduce the MIC of Cip against resistant bacteria. In addition, effects of Amp were indifferent also, but its effect loses that of Cip. Several studies have indicated that there is a synergistic effect of most of the extract of medicinal plants when used with antibiotics. Some studies have also concluded that it is possible to benefit from the medical plants' extracts in improving the antibacterial activity of some antibiotics that have lost their ability to control the infection caused by multidrug-resistant bacteria.^[19,20]

The inhibitory effects of the molasses, contribute to contain phenolic compounds that have high efficacy against microorganisms because of their own hydroxyl groups. The inhibition capacity of phenolic compounds increases by increasing these total compounds. Phenolic compounds inhibit cell growth by influencing cellular cell activity and thus depositing cell protoplasm.^[20] In addition, the molasses act on denaturation and break down the proteins of microorganism. Therefore, it can be considered as one

of the most important natural substances with clear antibacterial effects.^[21]

CONCLUSION

The main conclusion of this study is the dual use of molasses and antibiotics against *E. aerogenes* bacteria which will increase their lethal effectiveness of these pathogens, which will help overcome the problem of infection caused by multidrug-resistant pathogens. These results open the way of the other studies to show mechanical work of this combination.

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