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# Synergistic and antimicrobial effects of leaves oil (phoenix dactylifera) agonist gram negative bacteria

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## ABSTRACT

Antibiotic resistance is a significant issue that represent a global challenge. Hence, it has been urgent and crucial to find suitable interventions to control the spread of antibiotic resistant bacteria. Plants, on the other hand are considered as the main alternative to enhance or replace drug candidates. One of them in this context is the oily palm leaves methanolic extract. It has been found to exert antioxidant properties owing to its constituents (phenolic compounds) such as tannins and flavonoids. The study involved the determination of MIC and MBC along with the synergism effect induced by a range of concentrations of leaves oil. For E. coli, the MBC value was at a concentration of 100 mg/ml while highly resistant E. coli in concentration 50, 25, 12.5 and 6.25mg/ml. For salmonella the MBC value was at a concentration of 50 mg/ml while MIC at 200mg/ml. it can be concluded that the combination of leaves oils with antibiotics can be effective against resistant strains to traditional antibiotics.

**Keywords:** leaves oil, synergism, antibacterial, MIC, MBC, gram negative bacteria.

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## INTRODUCTION

At the practical level, any newly designed antibiotic is more likely to undergo bacterial resistance (WHO, 2014). Subsequently, there has been a growing effort to find alternative ways that improve the antibiotic activity and reduce bacterial resistance. Plants, on the other hand are considered as the main alternative to enhance or replace drug candidates. One of them in this context is the oily palm leaves methanolic extract. It has been found to exert antioxidant properties owing to its constituents (phenolic compounds) such as tannins and flavonoids (Abdallah, 2011).

The plant Phoenix dactylifera L is equatorial and semitropical tree that is also called "dates palm". It belongs to the family "Palmae", one of the oldest plants cultivated by human (Qadoos et al 2017). It is of economic importance in countries such as Iraq, Egypt, Emirates and Saudi Arabia (Alyousuf and Nikpay, 2020). In addition to be used for human and animal consumption, the products of dates palm are sometimes used as antimicrobial (Al-Shwyeh, 2019 ;Sahyon and Al-Harbi, 2020) and antiviral (Jassim and Naji, 2010). It has been shown that the dates palm leaves contain phenolic compounds that act as natural antioxidants and free radical scavengers and minimize the oxidative damage to lipids, proteins and nucleic acids (John and Shahidi, 2019). It has also been found that there is a considerable correlation between the total phenolic content (TPC) and antioxidant activities of the nonvolatile extracts (Hossain et al.,2011). In addition, certain phenolic compounds have shown activity against certain types of cancer (Khallouki et al., 2018; Rahaiee et al., 2020), anti-inflammatory effects (John and Shahidi, 2019) and cardiovascular diseases (Qadoos et al., 2017).

Among the main components of the oily palm leaves are flavonoids (as a main constituent) in addition to tannins, coumarins, , alkaloids, saponins, terpenoids, steroids, and carbohydrates (Suhaila, 2014). The inhibition potential of plant extracts against the growth of microbes was attributed to the presence of antioxidants Palm oil, like all fats, is composed of fatty acids, esterified with glycerol. Palm oil has an especially high concentration of saturated fat, specifically the 16-carbon saturated fatty acid, palmitic acid, to which it gives its name. (Toroglu, 2007; Lai et al 2015)

Finally pharmacological studies conducted on Phoenix dactylifera indicate the immense potential of this plant in the treatment of microbial and viral infections, gastric ulcer, diarrhea, skin disorders, cardiovascular disorder, and inflammatory ailments including liver and kidney disorders, , cancer (Baliga et al., 2011).
**This study aimed to** evaluate the synergism effects and MIC, MBC of leaves oil of Phoenix dactylifera ethanol extract on *Escherichia coli* and salmonella.

## MATERIALS AND METHODS

### Collection of plant material

Fresh leaves of Phoenix dactylifera, from Najaf cultivar, were washed thoroughly in running tap water, then the leaves were separated, cut into smaller pieces and dried for four week at room temperature .The dried leaves were blended to fine powder using a mechanical blender, that used for oil extraction

### Leaves oily extraction

Oily extraction for leaves powder of *Phoenix dactylifera* was done by transporting 20 g to special cellulose thimble that placed in Soxhlet apparatus the extraction chamber fitted with a condenser. Sample was placed on a 500-ml distillation flask containing 250 ml of organic solvents hexane then heated at 45ºC for period 5- 6 hours under reflex (10– 12cycles/h). After that the extracted solvent was evaporated using a Rotary vacuum evaporator at a temperature 40 °C with 90 rpm. The yield of oily extracts was expressed as a percentage of the

weight of extracts obtained from extraction relative to the weight of leaves powder used for extraction and then the oils obtained stored in refrigerator till analyzed according to Charef, *et al,* (2008).

$$\text{Yield of oily extraction} = \frac{\text{Weight of oil extract}}{\text{Weight of leaves powder used}} \times 100\%$$

**Isolation of pathogenic strains**

Pathogenic bacterial strain isolate using Eosin Methylene Blue (EMB) ,MacConkey, and Nutrient agar plate. The isolated strains were identified using, Indole, motility, urase agar (IMU) , Himedia, Simmons citrate test and kliglears iron agar test (IKA). Strain were chosen (*Sallmonllaenterica*, ,*Escherichia coli*), isolate from the clinical cases of Al-Forat Hospital/ Kufa.

**Antibiotic Sensitivity testing**

Bacterial strains turbidity was measured according to 0.5 MacFarland standards for $10^7$ cfu/ml. The bacterial strains were cultured on Mueller-Hinton agar (HIMEDIA, India), six different commercial antibiotic disks were spread on the agar and they included ciprofloxacin (CIP), amikacin (AK), trimethoprim (TMR), nitrofurantoin (F) ,doxycycline (DO),cefotaxime(CTX).disks, at stander concentration and pressed lightly onto the agar surface then overnight incubation at 37°C , the zone of inhibition (ZI) was measured for each bacterial growth around each disc .and results were set according to Clinical and Laboratory Standards Institute CLSI (2017) (Ahsan et al., 2015)

**Determination of MIC and MBC assay methods**

A serial dilution of stock Leaves oily extraction at concentrations 200 mg/ml and done was 6 tubes at different concentrations ( 6.25, 12.5, 25 ,50.100,200 mg/ml) were performed 1 ml of actively growing culture of pathogenic bacteria at a concentration of ($10^7$ colony-forming unit/mL) were mixed with extract dilution in tubes at the laminar cabinet II .All the tubes were incubated at 37 °C for 24 hrs. MIC and MBC were determination by culturing strains on counting plate and colonies were counted after 24 hrs. of culturing. The result of MIC and MBC were calculated according to bacteria colonies counting plates 1 ≥ colony number considered as sensitive, 2≤ colony number as intermitted and 10 ≤ colony number consider as resistant .

**Determination of synergism effects of the combination of leaves oil and antibiotics**

The bacterial strains were cultured on Mueller-Hinton agar six different commercial antibiotic disks were spread on the agar they included ciprofloxacin (CIP), amikacin (AK),trimethoprim(TMR), nitrofurantoin (F) , doxycycline (DO), cefotaxime(CTX).To evaluate the synergistic effect of the combination of the leaves oils and antibiotics which are in the form of ready to use discs, 2 µL of each essential oil was saturated to the antibiotic disc to determine the zones of inhibition (Ahsan et al 2015). antibiotic disks then overnight incubation at 37°C , the zone of inhibition (ZI) was measured for each bacterial growth around each discard results were set according to Clinical and Laboratory Standards Institute CLSI (2017) (Al-zeiny, 2018).

## RESULTS AND DISCUSSION

**Antibiotic Sensitivity**

The results of sensitive antibiotic tests were done against two gram negative bacteria were Salmonella enterica showed resistant for ciprofloxacin (CIP), trimethoprim (TMR), doxycycline (DO), cefotaxime (CTX) amikacin (AK) ZI (12 mm), and intermediate for nitrofurantoin (F) ZI (14mm) as showed in proves past studies (Moawad et al., 2017; Kuang et al., 2018) but *E coli* showed resistant for ciprofloxacin (CIP), trimethoprim(TMR),doxycycline (DO), nitrofurantoin (F) and cefotaxime ( CTX)ZI (24mm), but sensitive for amikacin (AK ZI) (22mm) as showed in research of Doi et al., (2020).

The MBC and MIC results showed that leave oil extract was inhibiting the bacterial activity of salmonella enteric strain and E Coli. where the 1≥ colony number , therefore the strain of Salmonella enteric and E coli showed sensitivity to leave oil extract of phoenix dactylifera in concentration 200,100,50 mg/ml and no effect of low concentration where the strains resistance for leaves oil extract that showed 10 ≤ colony number, which agreed past studies (Al-Daihan and Bhat, 2012) found the used of different parts of the plant extracts (leaves, fruit, seed, and bark)showed antibacterial activity against most tested microorganisms

E. coli showed the MBC, value at concentration 200mg/ml while, MIC value at concentration 100mg/ml , antibacterial that not appeared in E.coli at concentrations 50 mg/ml and 25,12.5,6.25mg/ml .while salmonella showed the MBC,MIC value at concentration 50 mg/ml showing in table (1) and figure (1), which is in agreement with the report from the previous study by Al-Daihan and Bhat (2012) as they reported antibacterial activity from E. coli and salmonella.

**Table 1: A range of concentrations of leaves oil extract of *phoenix dactylefira* on study group bacteria**

| Microorganism | Concentration of leaves oil extract (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 200 | 100 | 50 | 25 | 12.5 | 6.25 |
| ***Salmonella enterica*** | S | S | S | R | R | R |
| ***E coli*** | S | S | R | R | R | R |

S-sensitive , R- resistant,


Figure -1- appearance the MIC and MBC of leaves oil extract on two strains

**Synergism effects of the combination of leaves oil and antibiotics**

In agreement with findings obtained by Sani et al. (2017), our findings showed that the combination of leaves oil of Phoenix dactylifera (12.5-200 mg/ml) with amikacin and also with nitrofurantoin led to a synergistic effect on salmonella. Therefore, it can be proposed that the incidence of infections by Enterobacteriaceae can be treated and/or reduced by Phoenix dactylifera.

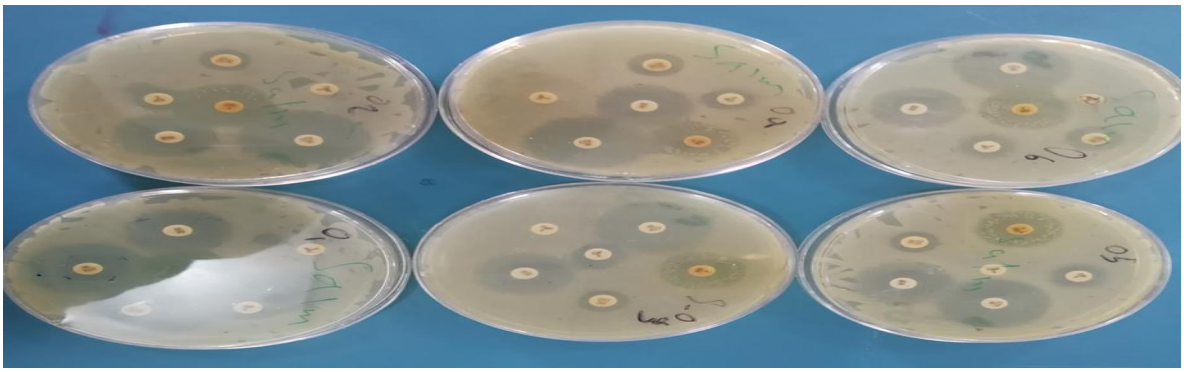
Showed a synergistic effect with leaves oil at (6.25 mg/ml), leaves oil Showed antimicrobial effects at different concentration as showed in table - 2 and figure-2.

On E coli leaves oil Showed a synergistic effect with amikacin at ( 6.25mg /ml ) but with other several concentration no effects on E.coli showed in table-3 and figure-3.

Table 2: Inhibition zones induced by a range of concentrations of leaves oil showing its antibacterial and synergistic effect with antibiotics on salmonella.

| mg/ml con . | CTP | CTX | F | TMP | AKS | DO |
|---|---|---|---|---|---|---|
| 200 | S | S | R | R | S | S |
| 100 | R | R | R | R | R | S |
| 50 | R | R | R | R | R | S |
| 25 | S | S | R | R | R | S |
| 12.5 | S | S | I | R | S | S |
| 6.25 | S | S | S | R | R | S |

R-



resistant, S- sensitive, I- intermitted

Figure 2: Inhibition zones induced by a range of concentrations of leaves oil showing its antibacterial and synergistic effect with antibiotics on salmonella.

Table 3: Inhibition zones induced by a range of concentrations of leaves oil of showing its antibacterial and synergistic effect with antibiotics on *E coli.*

| Concentration (mg/ml) | CTP | CTX | F | TMP | AKS | DO |
|---|---|---|---|---|---|---|
| 200 | R | R | R | R | S | R |
| 100 | R | S | R | R | S | R |
| 50 | R | I | R | R | S | R |
| | | | | | | |
| 25 | R | R | R | R | S | R |
| 12.5 | R | R | R | R | S | R |
| 6.25 | R | I | R | R | S | R |

R-resistant, S- sensitive, I- intermitted

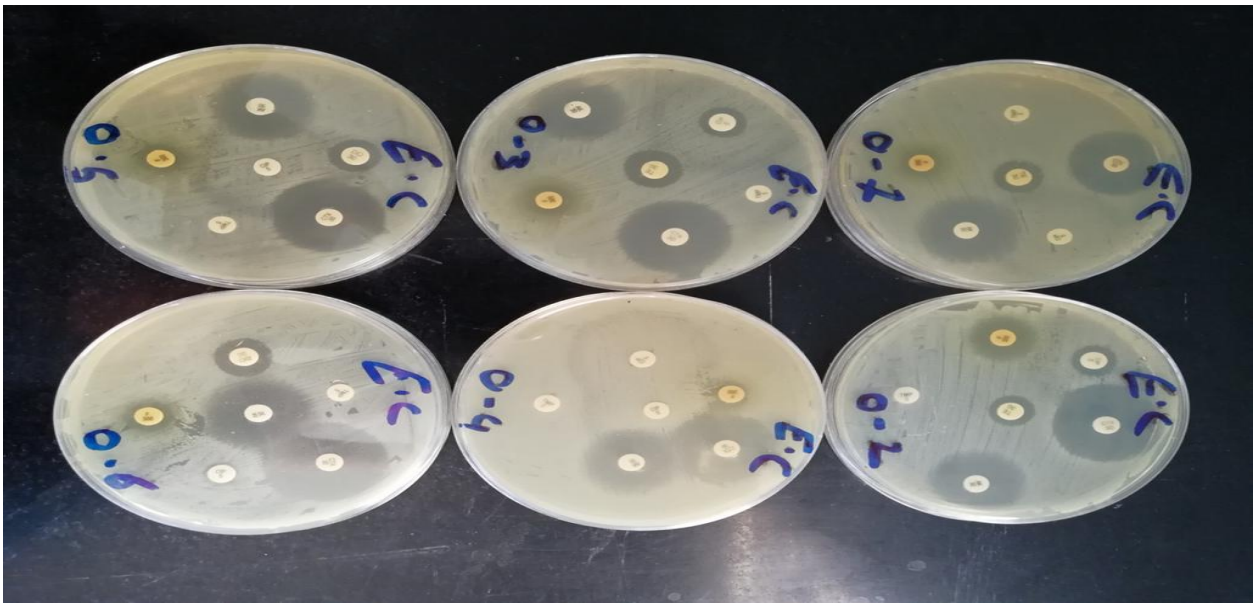


Figure 3: Inhibition zones induced by a range of concentrations of leaves oil showing its antibacterial and synergistic effect with antibiotics on *E coli*.

## CONCLUSION AND RECOMMENDATION

According to our findings, leaves oil exerts an antibacterial action whether by its own or in combination with antibiotics (i.e a synergistic effect) against emerging microbial drug resistance. Further investigation is recommended in terms of the effect of *leave oil* as an antibacterial agent against gram positive and gram-negative bacterial strains. In addition, we would recommend to investigate any potential antioxidant, anti-inflammatory, analgesic or anticancer effect of *leave oil*.

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