

Enhancing Antibacterial Activity Of Methanol Extract Of Phoenix Dactylifera Leaves Against Salmonella Entrica And Escherichia Coli Isolates

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Abstract

The present research detects the effectiveness and estimation of Methanol Extract of *Phoenix dactylifera* (leaves palm extract) as antibacterial activity against selected Gram negative bacteria (*Salmonella entrica* and *Escherichia coli*), which may be developed into new-fangled, harmless, and more efficient agents microbial infections. was determined by Serial doubling dilution of the plant extract in sterile nutrient broth to determine the minimum inhibitory concentration and achieved six different concentrations of 200µg/ml, 100µg/ml, 50µg/ml, 25 µg/ml, and 12.5µg/ml and 6.25 µg/ml, Its appeared as negative result (not have any zone of inhibition) in deferent concentration used.

When used the synergistic effect of Methanol Extract of *dactylifera* leaves against *Salmonella entrica* with different antibiotic showed Ciprofloxacin (CIP), Trimethoprim (TMP), resistance in deferent concentration but Cefataxime (CTX) sensitive in all concentration except (12.5 µg/ml, 6.250 µg/ml) were resistance. While Amikacin (AK) sensitive in concentration (50 µg/ml, 25 µg/ml) and Doxycycline (DO) sensitive in concentration (50 µg/ml) only.

Whereas the synergistic effect of *dactylifera* leaves against *Escherichia coli* were resisting in all antibiotics used except Cefataxime (CTX) sensitive in all concentration that used in the research and Amikacin (AK) sensitive in concentration (12.5 µg/ml, 6.250 µg/ml).

Key words: *E. coli*, *Salmonella*, Antibacterial effect, *Phoenix dactylifera*.

Introduction

The rare problem in the last three decades was an increased in number of multidrug resistant pathogenic bacterial strains which get an alarming in different countries [1]. There is a continuous need for the development of new antimicrobial drugs because the increase in number of drug resistant bacteria is no longer matched by discoveries of new drugs to treat infections [2]. Conferring by world health organization (WHO), medicinal plants can be a good source of variety of drugs [3].

In the Arabian world, in addition dates is a major fruit tree, the dates palm are a vital element of the daily diet, which is belongs to the family "Palmae", one of the oldest plants [4]. The phenolic profile of the plant revealed the presence of mainly cinnamic acids, flavonoid glycosides and flavanols and act as natural antioxidants, free radical scavengers, minimize the oxidative damage to lipids, proteins and nucleic acids [5]; therefore, the products of dates palm may be used as antimicrobial agent [6].

In Iraq, the number of trees became decreased to 16 million in last decades instead of 32 million in 1960 because of alternate wars, great number of palm canker and other various reasons [7]. A large number of pruned of dates palm eradicated and left portion became a waste every year, thus some researcher were recommended the possible uses of dates palm as sources of the antimicrobial properties of some pharmaceuticals materials as well as avoid multidrug resistant effect [8, 9]; therefore, the present research focus on detecting the effectiveness and evaluation of *Phoenix dactylifera* (date palm extract) methanol extract as antibacterial activity in vitro against *Salmonella entrica* and *Escherichia coli* as samples of Gram negative bacteria which may be developed into newfangled, harmless, and more efficient agents microbial infections.

Materials and Methods:

Isolation of Pathogenic Strains: Pathogenic bacterial strains were chosen (*Sallmonlla enterica*, *Escherichia coli*), isolate from the clinical cases of Al-Sadder Medical City/ Al-Najaf, Iraq according to manual and biochemical identification culture by using Gram's Stain, MacConkey agar, Xylose-Lysine Deoxycholate agar (XLD), Brilliant Green Agar (BGA) and Eosin Methylene Blue (EMB), Indole test, Catalase test, Oxidase test, Urease test, Simmons Citrate test and Methyl Red Vogues Proskauer media (MR-VP) [10] and confirmed the diagnosis by VITEK-2 compact system (bioMerieux/ France).

Antibiotic Sensitivity testing:

The bacterial strains were cultured on Mueller-Hinton agar and different antibiotic disks (concentration 10 µg) were spread on the agar which included amikacin (AK); Ciprofloxacin(CIP) ; Cefotaxime(CTX) ; Trimethoprim(TMP);Doxycycline (DO) disks at standard concentration. The zone of inhibition (ZI) was measured for each bacterial growth after overnight incubation at 37°C and results were set according to Clinical and Laboratory Standards Institute CLSI ,2017 [11].

Preparation of crude extract :

Collection, identification and preparation of date palm leaves Fresh samples of date palm leaves were collected from Kufa cultivar/Iraq, the leaves washed thoroughly in running tap water, then cut into smaller pieces and dried for four week at room temperature, blended to fine powder using a mechanical blender and dried leaves were powdered by using clean laboratory motor and pestle [12]. Fifty gram of fruit powder were crushed with 500 ml of methanol 70 % and mixed for 24 hrs in a magnetic stirrer at 45°C. The extract filtered and then concentrated under reduced pressure at 40 °C, 90 rpm using a rotary evaporator [13].

Bacterial Counting:

Twenty –five purely isolated colonies from fresh cultures were suspended in 5 ml of Nutrient broth .Turbidity resulting from growth was adjusted with sterile broth to give an optical density comparable to the 0.5 McFarland turbidity standard (equivalent to 1.5×10^8 colony forming units (CFU/ml).

Determination of antibacterial activity of the plant extract:

Isolated colonies inoculated with extract of *methanol leaves* , then culturing were spread-plate by swap on the account agar plate. The counting of the colonies of bacteria by colony counter after incubator for period 24 hrs. to assess the effect of extract of methanol leave against bacteria [14 ,15] , which the inoculated plates were incubated at 37°C for an overnight. Zones of growth inhibition were measured following 24 hours of incubation.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration :

Minimum Inhibitory Concentration (MIC) was determined by serial doubling dilution of the plant extract in sterile nutrient broth and achieved different concentrations of 200µg/ml, 100µg/ml, 50µg/ml, 25 µg/ml, and 12.5µg/ml, 6.25 µg/ml . Positive control containing nutrient broth plus organism while negative control containing nutrient broth plus plant extract only. All the test-tubes were incubated at 37°C for 24hours .

The result of MIC and MBC were calculated according to bacteria colonies counting plates $1 \geq$ colony number considered as sensitive, $2 \leq$ colony number as intermitted and $10 \leq$ colony number consider as resistant [13].

Synergistic Effect:

Determination of synergism effects of the combination of leaf extract and antibiotics was achieved serial doubling dilution of the plant extract in sterile distilled water at different concentrations of 200µg/ml, 100µg/ml, 50µg/ml, 25 µg/ml , 12.5µg/ml. and 6.25 µg/ml .

The bacterial strains were cultured on Mueller-Hinton agar , plant extract with different concentration and six different antibiotic disks were spread on the agar they included Ciprofloxacin (CIP), Amikacin (K), Trimethoprim (TMR), Cefataxime (CTX) , Doxycycline (DO) , then overnight incubation at 37° C , the zone of inhibition(ZI)was measured for each bacterial growth [16].

Results and Discussion

Antibiotic Sensitivity

The results of sensitive antibiotic tests were done against two gram negative bacteria , *Salmonella enterica* and *Escherichia coli* , in case of *S.enterica* indicated a varied levels of resistances for all antibiotic drugs that used in the present study [Ciprofloxacin (CIP), Trimethoprim (TMP), Doxycycline (DO), Cefotaxime (CTX) Amikacin (AK)]. While *E. coli* displayed resistant for all antibiotics [Ciprofloxacin (CIP), Trimethoprim (TMR) ,Doxycycline (DO) and Cefotaxime (CTX) except Amikacin (AK) was sensitive . As showed in corroborates previous studies in research of [13, 17,18].

The current study displayed that crude dates palm leaves checked the growth of both bacterial isolates, it has been reported that methanol extracts of the *P. dactyliferous* leaves not appear any effect on both bacterial in all concentration were used .

While MIC and MBC of the **Methanol leaves extract** as appear negative results (not have any zone of inhibition) in deferent concentration used (200µg/ml, 100µg/ml, 50µg/ml, 25 µg/ml, and 12.5µg/ml, 6.25 µg/ml) for *S. enterica and E.coli* as in Figure -1 .



Figure -1: Negative Results of MIC & MBC of the Methanol leaves extract for *S. enterica* and *E. coli*.

Secondary metabolites isolated from plants have been reported to possess antimicrobial properties [21]. The present results differed from preceding studies like [13, 16, 19] which found the use of different parts of the plant extracts showed antibacterial activity against most tested microorganisms. Some researchers confirmed that ethanol extracts of the *P. dactyliferous* leaves moderately inhibited the growth of Gram negative bacteria only [20, 22, 23]. *S. pyogenes* was confirmed as the most sensitive pathogen to the crude extracts and revealed the maximum zone of inhibition for methanol leaves, pits extracts, acetone leaves and pits extracts [8, 26].

Synergistic Effect:

Synergistic effect of Methanol leaves extract and antibiotic appeared as an inhibition zone of bacterial isolates against various concentrations of extraction (200µg/ml, 100µg/ml, 50µg/ml, 25 µg/ml, and 12.5µg/ml, 6.25 µg/ml for *S. enterica* and *E. coli* (Figure -2: A ; B) with various antibiotics.

Synergistic effect of Methanol Leaves against *S. enterica* with different antibiotics showed Ciprofloxacin (CIP); trimethoprim (TMP) were resistance in different concentrations but Cefotaxime (CTX) was sensitive in all concentrations except (12.5 µg/ml; 6.250 µg/ml) its resistance. Amikacin (AK) was sensitive in concentration (50 µg/ml ; 25 µg/ml) while Doxycycline (DO) sensitive in concentration (50 µg/ml) only as in table-1.

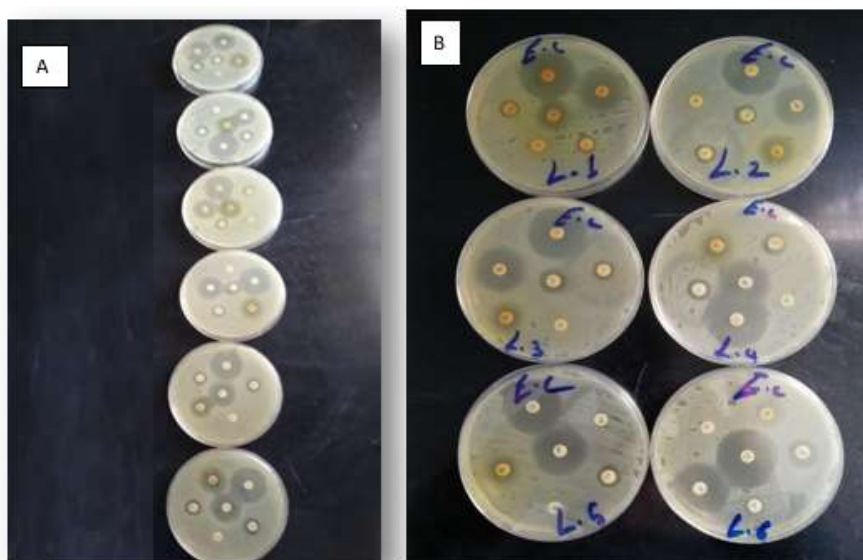


Figure - 2 : Synergistic Effect of Methanol Dactylifera Leaves Extract Against Selective Bacteria
A: *Salmonella enterica* ; **B:** *Escherichia coli*

Whereas synergistic effect of Methanol Leaves against *E. coli* with different antibiotics showed Cefotaxime (CTX) was sensitive in all concentrations but Amikacin was sensitive in concentration (12.5 µg/ml; 6.250 µg/ml) and all other concentrations were resistance as in table-2.

Table -1: Synergistic Effect of Methanol Dactylifera Leaves Extract against *Salmonella enterica* with Different Antibiotic.

Parameters/ *** Abx	CIP	CTX	TMP	AK	DO
200	*R	**S	R	R	R
100	R	S	R	R	R
50	R	S	R	S	S
25	R	S	R	S	R
12.5	R	R	R	R	R
6.2	R	R	R	R	R

* R = Resistant ; **S = Sensitive ; *** Abx: antibiotic

Table -2 : Synergistic Effect of Methanol Dactylifera Leaves Extract against *E. coli* with Different Antibiotic .

Parameters/*** Abx	CIP	CTX	TMP	AK	DO
200	R	S	R	R	R
100	R	S	R	R	R
50	R	S	R	R	R
25	R	S	R	R	R
12.5	R	S	R	I	R
6.2	R	S	R	I	R

The current study demonstrated that methanol extracts of the *P. Dactyliferous* leaves give a strong synergism affect with various antibiotics [8].

Antimicrobial activity by date palm leaves extracts against numerous pathogens responsible for wide variety of infections which might be due to the selective or synergistic action of various chemicals present in date palm leaves [22]. Additionally, presence of antimicrobial activity in whole date plant may be consider as defense tool of plants against an array of microbes [25, 26] . The methanol extract of Phoenix dactyliferous leaves was inhibitory effect on the growth of *S. aureus* and *B. subtilis* but resistance to *P. aeruginosa* and *E. coli* [24].

Conclusion

The methanol extract of Phoenix dactyliferous leaves demonstrated not have any antibacterial affect against *Salmonella enterica* and *Escherichia coli* but have an obvious antibacterial activity in vitro against studied bacteria with antibiotic synergism .

Recommendation

In future research will study the efficiency of used nanoparticles with menthol extract in different concentration and the effectiveness on genetic structure of the studied bacteria , use a higher concentration to give better results and application on lab animals.

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