Antibacterial activity of *Phoenix dactylifera* L. leaf extracts against several isolates of bacteria

Biologist, Nuha Qays Abdul Maged  
Biologist, Nadheema Abed Abbas

Faculty of Veterinary Medicine, University of Kufa

Abstract:
The objective of this research is to find a natural chemical compounds alternative available and free of side effects and high-impact effective against many bacterial species. The crude ethanol extracts of *Phoenix dactylifera* leaves were tested for antibacterial action against bacterial isolates, the ethanol extracts showed good antibacterial activity against all isolates *Enterobacter* sp., *Salmonella* sp., *Pseudomonas aeruginosa* *Escherichia coli*, *Staphylococcus aureus*, *Klebsella pneumonia*, *Streptococcus pneumoniae*, and *Protus* sp. bacterial isolates which had shown zone of inhibition against all plant extracts concentration with exceptions at 25 mg/ml concentration related with *Salmonella* sp., *Pseudomonas aeruginosa*, *Escherichia coli* and *Protus* sp., also the largest inhibition zone were revealed at 100 mg/ml concentration especially with *Escherichia coli* and *Streptococcus pneumoniae* isolate while *Salmonella* sp. isolate exhibited the smallest inhibition zone under 50mg/ml concentration.

Introduction:
Date palm *Phoenix dactylifera* L. is a major fruit tree in most of Arabian countries and it is considered one of the most important commercial crops. Dates, the fruits of date palm are a vital element of the daily diet in the Arabian world.
Phytochemically, the whole plant contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins, the phenolic profile of the plant revealed the presence of mainly cinnamic acids, flavonoid glycosides and flavanols (4,5,6). The number of trees in Iraq is become decreased there are 32 million in 1960 and 16 million in last decades. This is Assign to a alternate wars, preponderance of palm canker and other various reasons (7). Every year about three million dates palm are pruned and this leaves portion becomes a waste, this attracted us for its use as a research material for analyzing antibacterial activity, initially as a control project, some researcher were suggested the potential uses of dates palm as sources of pharmaceuticals materials (8).

During the last three decades the problem of antimicrobial resistance was getting increasingly acute and an increase in number of multidrug resistant pathogenic bacterial strains has grown at an alarming rate in different countries, 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 (9).

According to World Health Organization, medicinal plants can be a good source of variety of drugs. As a preventive and curative measure, plants and their products have been used in the treatment of infections for centuries. WHO estimated that 80% of the people worldwide rely on plant based medicines for their primary healthcare (10). As the global interest towards traditional medicines over the conventional treatment is increasing due to their safer action (in terms of tolerance and side effects) for chronic illnesses, this study was achieved to evaluate the antimicrobial properties of some cultivars of date palm of Iraq, which may be developed into new, safer, and more efficacious agents to combat serious microbial infections.

Materials and Methods:
Collection and storage of plant samples:
Fresh leaves were evaluated for antibacterial activity. Fresh samples were labeled and stored at 4°C. The fresh leaves were washed individually under running tap water to remove soil particles and other dirt. The leaves were air dried in the laboratory at room temperature (30 ± 2°C) for 7 days. The dried leaves samples were ground well into a fine powder with the help of mill and a mixer grinder respectively. The powder was stored in air sealed plastic containers at room temperature till extraction was carried out.

Extraction:
The common method (1) with some modification was adopted for preparation of plant extracts. Briefly, 10 g of powdered plant material was soaked separately in 100 ml distilled water and ethanol for 72 h. Each mixture was stirred at 24 h interval using a sterile glass rod. At the end of the extraction, each extract was passed through What man No. 1 filter paper. The filtrate obtained was reduced to dryness by removing solvent in air dried oven at 40°C. Each dried crude extract was dissolved in 2 ml distilled water and stored in eppendorf tubes at -18°C till antimicrobial activity was performed.

Determination of antimicrobial activity of the plant extract
Test organisms
Bacterial isolates Enterobacter sp., Salmonella sp., Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsella pneumoniae, Streptococcus pneumoniae, and Proteus sp. (clinical isolate) were obtained from the patients, were recruited from the laboratories department in Al-Sadder Medical City in Al-Najaf Government. Strains of bacteria were maintained on nutrient broth media.
Antibacterial assay

The crude extracts were screened against various human pathogens by agar well diffusion (11). In this method, 10 ml aliquots of nutrients broth was inoculated with the test organism and incubated at 37°C for 24 h. Sterile cotton swabs were dipped in the bacterial suspension and evenly streaked over the entire surface of the Muller Hinton agar plate to obtain uniform inoculums. Three wells per plate were made with the sterilized pasture pipette. Crude extract (50 μl) was poured in respective wells with the help of micropipette. Penicillin and Gentamycin was used as positive control. Each extract was analyzed in duplicate. All the plates were incubated for 24 h at 37°C. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest (mm) as observed from the clear zone surrounding the well.

Results:

The antibacterial activity of Phoenix dactylifera L. leaves crude extracts were evaluated against eight of bacterial isolates. The ethanol extracts had shown good antibacterial activity against all tested bacteria (Tables 1 and Figure 1), data zone of inhibition indicates that these extracts were able to check the growth of all bacterial isolates which had shown zone of inhibition against all plant extracts concentration used in the study with some exceptions at 25 mg/ml concentration related with Salmonella sp., Pseudomonas aeruginosa, Escherichia coli and Protus sp. which had not shown any zone of inhibition. In general the effect of extract was elevated when the concentration increased gradually to 100 mg/ml (Tables 1 and Figure 1).

Generally the present study also revealed the largest inhibition zone at 100 mg/ml concentration especially with Escherichia coli and Streptococcus pneumoniae isolate which is about 14 mm, in the other hand Salmonella sp. isolate exhibited the smallest inhibition zone 6 mm especially under 50mg/ml leave palm extracted concentration (Figure 1).

Table 2. Antibacterial activity of crude extract of Phoenix dactylifera L.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Leaves palm extract (mg/ml)</th>
<th>Positive control (mg/ml)</th>
<th>Inhibition zone (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>9</td>
<td>9.5</td>
<td>11</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>-</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>11</td>
<td>11.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>9</td>
<td>10</td>
<td>10.5</td>
</tr>
<tr>
<td>Klebsella pneumonia</td>
<td>8</td>
<td>9</td>
<td>10.5</td>
</tr>
<tr>
<td>Streptococcus pneumoniaae</td>
<td>8</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>Protus sp.</td>
<td>-</td>
<td>11</td>
<td>11.5</td>
</tr>
</tbody>
</table>

* Data are the average of two experiments. - = Not active against tested bacteria.
Discussion:
Numerous plants and secondary metabolites isolated from plants have been reported to possess antimicrobial properties (12,13,14,15,16). Results of the present study showed that crude extract of Dates palm leaves checked the growth of all bacterial isolates with some exception (Tables 1). Earlier it has been reported that ethanol extracts of the *P. dactylifera* leaves moderately inhibited the growth of Gram positive and Gram negative bacteria (17,18). Furthermore, the leaves extracts

Figure(1): Inhibition zone of bacterial isolates against various concentrations of date palm leaves extraction (25, 50, 75 and 100 mg/ml), A: Protues sp., B: Pseudomonas aeruginosa, C: Enterobacter sp., D: Streptococcus pneumonia, E: Staphylococcus aureus.
have shown promising antibacterial activity against Streptococcus pneumonia.

The phytochemicals derived from root, stem, leaves, fruits, flowers and seeds of medicinal plants include phenolics compounds, essentials oils, proteins and antioxidants, together they work as biocontrol agents (19). The inhibition potential of plant extracts against the growth of microbes was attributed to the presence of antioxidants (20,21). It has been reported that the whole date plant (including pits and leaves) contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. The phenolic profile of the plant revealed presence of mainly cinnamic acids, flavonoid glycosides, flavanols, four free phenolic acids and nine bound phenolic acids (22,23,24,25,26).

The results of our study demonstrated excellent antimicrobial activity by date palm leaves extracts against various 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. Furthermore, presence of antimicrobial activity in whole date plant may be considered as defense tool of plants against an array of microbes. This seems important for better yield of the dates of immense commercial value in the Kingdom.

References:


