

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.

استعمال مستخلص سعف النخيل . Phoenix dactylifera L كمضاد ميكروبي ضد عدد من العز لات البكتيرية

البيولوجي نهى قيس عبد المجيد البيولوجي نظيمه عبد عباس جاسم

كلية الطب البيطري، جامعة الكوفة

الخلاصة:

الهدف من هذا البحث هو إيجاد مركبات كيميائية طبيعية بديله متوفرة وخاليه من التأثيرات الجانبية وذات تأثير فعال ضد العديد من الأنواع البكتيرية . اذ تم في الدراسة الحالية اختبار نشاط المستخلص الكحولي الخام لورق نخيل التمر كمضاد بكتيري تجاه بعض العزلات البكتيرية، إذ اظهر المستخلص نشاطاً جيداً اتجاه جميع العزلات البكتيرية Enterobacter sp., Salmonella sp., Pseudomonas aeruginosa, Escherichia coli, Staphylococcus Enterobacter sp., Salmonella sp., Pseudomonas aeruginosa, Escherichia coli, Staphylococcus البكتيرية مناطق منع للنمو اتجاه كما تراكيز المستخلص النباتي مع بعض الاستثناءات المتعلقة بالتركيز 25 ملغم/مل البكتيرية مناطق منع للنمو اتجاه كافة تراكيز المستخلص النباتي مع بعض الاستثناءات المتعلقة بالتركيز 25 ملغم/مل منع للنمو ظهرت عند تركيز 100 ملغم/مل وخصوصاً للعزلتين Escherichia coli منع للنمو ظهرت عند تركيز 100 ملغم/مل وخصوصاً للعزلتين ويزاد ورفي دوست ويوسما البعريز مناطق منع الغرابه. وعاليه من العزائي في المعربة من العربي المعار المعالية المعربة المعارفة من المعالية المعربة العربي العربي مناطق ويوسم المعاربي مناطق منع المواتجاه كافة تراكيز المستخلص النباتي مع بعض الاستثناءات المتعلقة بالتركيز 25 ملغم/مل ومناطق منع النمو المعاربة المعاربة مراطق النباتي مع معن الاستثناءات المتعلقة بالتركيز 30 ملغم اللعزائين العزلات و هربي مناطق منع النمو تجام العربي المعاربة مراطق منع النمو عند تركيز 100 ملغم/مل وخصوصاً للعزلتين المعاربة من المعاربة مراطق معربي المعربة العربي المعاربة المعاربة المعاربة مراطق منع النمو عند تركيز 100 ملغم/مل وخصوصاً العزلين وعملي من النمو عند تركيز 100 ملغم/مل المعالية مناطق من المعاربة ومراطق مراطق المعاربة مراطق من مراطق من المعاربة المعاربة المعاربة مراطق من المعاربة مراطق من المعاربة المعاربة مراطق المعاربة من المعالية من المعالية من المعالية من المعاربة المعاربة مراطق اللمورت ألعرات المعربة مراطق من من المعاربة من من المعاربة من المعاربة مراطق من مراطق من المعاربة المعاربة المعاربة مراطق من من من من من مراطق من من من من مولي من مراطق من من المعاربة مراطق من مراطق من من المعاربة مربة مراطق مربوبة مراطة من من من من من من مربة مراطق من من من من مربقة من من من من من من من مربة مراطق من من من من مولي م

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 (1,2,3). 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 number of multidrug resistant in 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 interest towards traditional global 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

Vol. (4) No. (2) 2013

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 $(30 \pm 2^{\circ}C)$ 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., Pseudomonas Salmonella sp., aeruginosa, Escherichia coli. *Staphylococcus* aureus, Klebsella pneumoniae, Streptococcus pneumoniae, (clinical isolate) were and *Protus* sp. obtained from the patients, were recruited from the laboratories department in Al-Medical City Sadder 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).

Bacterial isolates	Leaves palm extract (mg/ml)				Positive	control
					(mg/ml)	
	25	50	75	100	Gentamicin	Penicillin
	Inhibition zone (mm)*					
Enterobacter sp.	9	9.5	11	12	25	20
Salmonella sp.	-	6	9	11	28	23
Pseudomonas aeruginosa	-	11	11.5	13	25	20
Escherichia coli	-	11	13	14	30	34
Staphylococcus aureus	9	10	10.5	11	22	23
Klebsella pneumonia	8	9	10.5	12	28	20
Streptococcus	8	10	12.5	14	27	21
pneumoniae						
Protus sp.	-	11	11.5	13	30	25

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

* Data are the average of two experiments . - = Not active against tested bacteria .



leaves extraction (25,50,75 and 100 mg/ml) ,A:Protues sp.,B: Pseudomonas aeruginosa, C: Enterobacter sp., D: Streptococcus pneumonia ,E: Staphylococcus aureus.

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

Vol. (4)

2013

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:

1-Duke, J.A. (1992). Handbook of phytochemical constituents of GRAS herbs and other economic plants. CRC Press, Boca Raton.

2-Vayalil, P.K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. Arecaceae). J. Agric. Food Chem., 50: 610-617.

3-Tahraoui, A., El-Hilaly, J., Israili, Z.H. and Lyoussi, B. (2007). Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (*Errachidia province*). J. Ethnopharmacol., 110: 105-117.

No. (2)

4-Seelig, R.A. (1974). Fruits and vegetables facts and pointers. United fresh fruit and vegetable association, Washington, DC, USA.

5-Dowson, V.H.W. (1982). Date production and protection. FAO plant production and protection. Food and Agriculture Organization of the United Nations, 35 p.

6-Biglari, F., Abbas, F.M.,and Azhar, M.E. (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem., 107: 1636-1641.

7- البدري، باسم حازم (2010). دراسة اقتصاية حول واقع انتاج التمور في العراق (2008-2009). مجلة الشجرة المباركة. المجلد 2. العدد 1. الصفحه 26-38.

8-Al-Shahib, W., Marshall, R.J. (2003). Fatty acid content of the seeds from 14 varieties of date palm *Phoenix dactylifera* L. Int. J. Food Sci. Technol., 38: 709-712.

9-Whitman, T.J. (2008). Communityassociated methicillin - resistant *Staphylococcus aureus* skin and soft tissue infections. Dis. Month, 54: 780-786.

10-Alagesaboopathi, C. (2011). Antimicrobial screening of selected medicinal plants in Tamilnadu, India. Afr. J. Microbiol. Res., 5: 617-621.

11-Khan, M.A., Inayat, H., Khan, H., Saeed, M., Khan, I., and Rahman, I. (2011). Antimicrobial activities of the whole plant of *Cestrum nocturnum* against pathogenic microorganisms. Afr. J. Microbiol. Res., 5: 612-616.

12-Ali, H., Qaiser, M. (2009). The ethnobotany of Chitral valley, Pakistan

with particular reference to medicinal plants. Pak. J. Bot., 41: 2041.

13-Qadrie, Z.L., Jacob, B., Anandan, R., Rajkapoor, B.and Ulla, M.R. (2009). Antibacterial activity of ethanolic extract of *Indoneesiella Echioides* (1) Nees. evaluated by the filter paper disc method. Pak. J. Pharm. Sci., 22: 123-125.

14-Nizar, M.A., El Sukhon, S., Msameh, Y., and Abdul-Karim, J.S. (1999). Effect of date fruits, *Phoenix Dactyliferia* L., on the hemolytic activity of Streptolysin O. Pharma. Biol., 37: 335-339.

15-Nisar, M., Qayum ,M., Shah, M.R., Kaleem, W.A.,and Ali, I. (2010). Antimicrobial screening of impatiens bicolor royle. Pak. J. Bot., 42: 523-526.

16-Samiullah, B.A., Bano, A., Naz, R.and Yasmin, H. (2011). *In vitro* inhibition potential of *Lespedeza bicolor* Turcz against selected bacterial and fungal strains J. Med. Plant. Res., 5: 3708-3714.

17-Sabah, A., Jassim, A., and Mazen, A. N. (2007). *In vitro* evaluation of the antiviral activity of an extract of Date Palm (*Phoenix dactylifera* L.) pits on a *Pseudomonas* Phage. eCAM, pp. 1 - 6 doi:10.1093/ecam/nem160.

18-Ammar, N.M., Lamia, T., Nabil, H., El-Sayed, M., and Tom JM (2009). Flavonoid constituents and antimicrobial activity of date (*Phoenix dactylifera* L.) seeds growing in Egypt. In: Proceedings of 4th conference on research and development of pharmaceutical industries (Current Challenges). Med. Arom. Plant Sci. Biotechnol., 3: 1-5.

19-Cragg, G.M., Simon, J.E., Jato, J.G., Sander, K.M. (1996). Drug discovery and development at the National Cancer Institute: Potential for new pharmaceutical crops. Progress in new crops, ASHS Press, Arlington, pp. 554-560.

20-Cutter, C.V. (2000). Antimicrobial effect of herb extracts against *Escherichia coli, Listeria monocytogenes* and *Salmonella typhimurium* associated with beef. J. Food Prot., 63: 601-607.

21-Puupponen, P.R., Nohynek, L., Meiier, C., Kahkonen, M., Heinonen, M., Hopia, A., and Caldentey KM (2001). Antimicrobial properties of phenolic compounds from berries. J. Appl. Microbiol., 90: 494-507.

22-Alade, P.I., Irobi, O.N.(1993). Antimicrobial activities of crude leaf extracts of *Calypha wilknesiama*. J. Ethnopharmacol., 39: 171-174.

23-Eong, Y.J., Hong, F.A., Adel, A., Kader, S.,and Alyson, E. (2006). The flavonoid glycosides and procyanidin composition of Deglet Noor dates (*Phoenix dactylifera*). J. Agric. Food Chem., 54: 2405-2411.

24-Kahkashan, P., Najat, A. B. and Dina, A. W. (2012). Antibacterial activity of *Phoenix dactylifera* L. leaf and pit extracts against selected Gram negative and Gram positive pathogenic bacteria. Journal of Medicinal Plants Research Vol. 6(2), pp. 296-300.

25-Mosa, J.S., Hifnawy, M.S.,and Mekkawi, A.G. (1986). Phytochemical and biological investigations on date palm seeds (*Phoenix dactylifera* L.) produced in Saudi Arabia Arab. Gulf J. Sci. Res., 4: 495-507.

26-Ziouti, A.C., Modafar, E.L., Fleuriet, A.S., Boustani, E.L.and Macheix, J.J. (1996). Phenolic compounds in date palm cultivars sensitive and resistant to *Fusarium oxysporum*. Biologia. Plantarum, 38: 451-457.7.