Antibacterial effect of date palm (*Phoenix dactylifera L.*) pit aqueous extract on some bacteria cause urinary tract infection.

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Abstract

The present work was carried out to study the impact of date palm pit aqueous extract as an antibacterial on four species of pathogenic bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*). Four concentrations of date palm pit aqueous extract were prepared by using normal saline: 200,100,50 and 25(mg/ml).200 and 100(mg/ml) were the most effective concentrations on the tested bacteria except *K. pneumoniae* which showed full resistance to all extract concentrations. Date palm pit extract was somewhat more effective in inhibiting growth of bacteria as compared with antibiotics includes trimethoprime, gentamycin and rifampin.

Key words **:** urinary tract infection**,** date palm

تأثير المستخلص المائي لنوى التمر على بعض أنواع البكتريا المسببة لالتهاب المجاري البولية.

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الخلاصة

اجريت هذه الدراسة لغرض دراسة تأثير المستخلص المائي لنوى التمر كمضاد بكتيري على اربع انواع بكتيرية ممرضة ومسببة لالتهاب المجاري البولية وهي: *Escherichia coli* و *Klebsiella pneumoniae* و *Proteus vulgaris* و *Pseudomonas aeruginosa*.تم تحضير أربع تراكيز من المستخلص المائي لنوى التمر بإستعمال المحلول الملحي الفسلجي وهي: 200، 100 ، 50، 25(ملغم/مل).أظهرت النتائج أن التراكيز 200و100(ملغم/مل) كانت الأكثر فعالية في تثبيط البكتريا قيد الدراسة، في حين أن بكتريا *K. pneumonia* أظهرت مقاومة كاملة لكل تراكيز المستخلص المائي لنوى التمر. كان المستخلص المائي لنوى التمر الى حد ما اكثر فعالية في تثبيط البكتريا قيد الدراسة عند مقارنته مع فعالية المضادات الحيوية والتي تشمل الميثوبريم، الجنتامايسين والريفامبسين.

الكلمات المفتاحية : التهاب المجاري البولية ، نوى التمر

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Introduction:

The date palm (*Phoenix dactylifera L.*) is considered the most important source of food for humans in both arid and semiarid regions(1).The pits or seeds of many fruit are used in complementary and alternative medicine (CAM) in an effort to prevent illness, reduces stress, prevent or reduce side effects and symptoms, or control or cure diseases(2). The date pits, also called pips, stones, kernels or seeds, form part of the integral date fruit. Depending on variety and grade quality, the pits represent about 6–12% of the total weight of the mature date (3). Dates contain a high percentage of sugars reaching 88% in some varieties (4). Dates are also rich in mineral salts and vitamins (5). For the date pit, the percentage of non-reducing sugars is 3.82% and in glucose and fructose this is 1.68% and 1.53% respectively(6). The pits of *Phoenix dactylifera* contain different chemical compounds such as saturated and unsaturated fatty acids, Zinc (Zn), Cadmium (Cd), Calcium (Ca) and potassium (K). Saturated fatty acids include stearic and palmitic acid and unsaturated fatty acids contain linoleic and oleic acids which could inhibit the 5-α reducates enzyme(7). In terms of dry weight, the chemical composition of date pits has been reported as containing 5–10% moisture, 5–7% protein, 7–10% oil, 10–20% crude fiber, 55–65% carbohydrates and 1–2% ash (2,8). The carbohydrates, as the largest component of the dry weight, are typically comprised of neutral detergent fiber 75%, acid detergent fiber 57.5%, hemicelluloses 17.5%, lignin 11%, cellulose 42.5% and ash 4% (2,9). The seed powder is also used in some traditional medicines and has been investigated for human potential health benefits (9), and for addition to animal feed to enhance growth (10,11,12,13), the latter an action that has been ascribed to an increase in the plasma level of estrogens (14) or testosterone (15). Date pits have been studied as potential sources of edible oils and pharmaceuticals (16). In addition, date pit extract shows an ability to restore the normal functional status of the poisoned liver, and also to protect against subsequent carbon tetrachloride hepatotoxicity on the liver in rats (17). However, the antimicrobial activity of date pits has been poorly investigated (9), and only an ethanolic extract of date pits has been used and this only showed a weak antimicrobial activity on several strains of microorganisms (18),the evidence of the aqueous date palm pits extract that can be used as CAM to treat human bacterial diseases is discussed in present study.

Materials and Methods

-Preparation of *Phoenix dactylifera* (Date Palm) Pits extract:

The pits were collected,rinsed well and then they were roast until they were slightly burned.The dried pits were ground into a fine powder and immersed in normal saline (1:5 ratio, weight to volume) for 24hrs. at room temperature(17) .

-*Bacterial* Isolates:

Escherichia coli, *Klebsiella pneumoniae* , *Proteus vulgaris* and *Pseudomonas aeruginosa* were obtained from Department of Biology, College of Science, University of Baghdad after their isolation from patients infected with urinary tract infections and their diagnosis.

-Concentrations of date palm pit extract:

Four concentrations of date palm pit extract were prepared by using normal saline: 200,100,50 and 25(mg/ml) after preparing the stock pit extract solution by using normal saline for 24hrs. at room temperature.

-Effect of date palm pit extract *in vitro*:

The antibacterial activity was determined by using agar well diffusion method (19). $1x10^8$ CFU/ml bacterial concentrations were prepared for each bacteria in normal saline then wells were made in Muller Hinton agar by using the opposite side of micropipette tips.Plates were cultured by using small swab of each bacteria.100µl of pit extract was introduced into the wells from each concentration.The inoculated plates were incubated at 37C° for 24 hrs. The diameters of inhibition zones were measured for each plate. The standard cefotaxime and ceftriaxone discs(30mcg) were used as control for *E. coli*, standard chloramphenicol(10 mcg) and ciprofloxacin(5 mcg) discs were used as control for *K.*

pneumoniae, Ampicillin(10 mcg) and Trimethoprim(5 mcg) discs were used as control for *P. vulgaris* and finally Gentamycin(30 mcg) and Rifampin(5 mcg) discs were used as control for *P. aeruginosa* by pressing these discs on the cultured plates gently.

Results

The emergence of the inhibition zone of the growth of pathogenic *E. coli*, *K. pneumonia*, *P. vulgaris* and *P. aeruginosa* as a result of transactions with extract of pits of date palm is clear from Table 1.Results showed that 200mg/ml of pit extract was the best concentration followed by 100mg/ml for *E. coli*, *P. vulgaris* and *P. aeruginosa* inhibition, while no effect had been showed on *K. pneumoniae* at any concentration of date palm pit extract.On the other hand,50 and 25(mg/ml) of pit extract were not effect on any of the tested bacteria. The resulted inhibition zones formed around the wells(which were made by using the wide side of blue tips) were(17,15,13)mm for *E. coli*, *P. aeruginosa* and *P. vulgaris* respectively when using 200mg/ml, while the inhibition zone diameters when using 100mg/ml were(15,14,12)mm for *E. coli, P. aeruginosa* and *P. vulgaris* respectively.Results obtained showed that the impact of *Phoenix dactylifera* (Date Palm) Pits extract concentrations(200 and 100mg/ml)were more effective than the impact of anti-vital gentamycin and Rifampin which gave 9 and 10(mm)of inhibition zones when used to treat *P. aeruginosa.* Pit extract 200mg/ml concentration was more effect in inhibition of *P. vulgaris*(13mm) than the effect of antibiotics used against the same bacteria(Ampicillin and Trimethoprime),while 100mg/ml concentration showed the same inhibition diameter of Trimethoprim which was 12mm.For *E. coli*,Pits extract concentrations gave inhibition diameters(17,15mm) which were less than the impact of cefotaxime and ceftriaxone that showed 22 and 24(mm) of inhibition zones.Finally, *K. pneumoniae* showed full resistance towards all pit extract concentrations(Results showed in table 1 and 2). The appropriate recommendations in this study are to use nuclei dates antimicrobial on *E. coli*, *P. vulgaris* and *P. aeruginosa* than the activity of standard antibiotic and to make more studies to know pit extract efficacy on *K. pneumonia*.

Table 1:Inhibitory effect of pit extract against pathogenic bacteria.

Pit extract concentr-ations (mg/ml)	Inhibition zone diameters of Pit extract concentrations on *E. coli* (mm)	Inhibition zone diameters of Pit extract concentrations on *K. pneumoniae* (mm)	Inhibition zone diameters of Pit extract concentrations on *P.* vulgaris (mm)	Inhibition zone diameters of Pit extract concentrations on *P. aeruginosa* (mm)
200	17	-	13	15
100	15	-	12	14
50	-	-	-	-
25	-	-	-	-

(-): No inhibition.

Table 2: Sensitivity of bacterial isolates for antibiotics.

Bacteria	Antibiotics(mcg)	Diameter of nhibition (mm)
E. coli	Cefotaxime (30)	22
	Ceftriaxone (30)	24
K. pneumonia	Chloramphenicol(10)	-
	Ciprofloxacin(5)	22
P. vulgaris	Ampicillin (10)	-
	Trimethoprime (5)	12
P. aeruginosa	Gentamycin (30)	9
	Rifampin (5)	10

(-): No inhibition.

Discussion

From the obtained results,it was clear that 200 and 100(mg/ml) of date palm pit extract had a significant antibacterial activity on *E. coli, P. aeruginosa* and *P. vulgaris* while *K. pneumoniae* was not affected by any pit extract concentrations. Date palm pit extract was somewhat more effective in inhibiting growth of most tested bacteria as compared with antibiotics and this may refer to differences in resistance of bacteria to anti-tested materials due to change in membrane permeability of cells, thereby hindering the entry of enzymes or excretions by the change in the chemical composition of the constituent chemical or by changing the nature of some of their components(20).On the other hand,the resistance of *K. pneumoniae* to the pit extract may refer to the presence of capsule which consider one of the virulence factors that *K. pneumoniae* posses and make this bacteria show some antibiotics resistance. The pits of date palm (*Phoenix dactylifera L.*) are an inexpensive component found in abundance in the Middle East(2). In this study, date palm pit extract has demonstrated positive antibacterial results.

The bacterial inhibition may be attributable to date pit extract's heat-labile bioactive component(s) attaching to or modifying the surface of the bacteria. The bioactive

component(s), namely, protein and some derived polyphenolic compounds such as polysaccharides, lignans and bioflavonoids, are present in reasonable amounts in date pit (3,21), which were reported to act principally by binding to the protein. (22).The results obtained from this study seems agreement with(23,24). It is reasonable to conclude that a further phytochemical characterization of the active ingredients may reveal useful compounds, and may also provide the basis for further refinement of antibacterial drug design and development as potential biotherapeutic agents against medically-important bacteria cause urinary tract infections.

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