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Full Length Research Paper

Antibacterial activity of *Phoenix dactylifera* L. leaf and pit extracts against selected Gram negative and Gram positive pathogenic bacteria

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The crude methanol, acetone and water extracts of leaves and pits of three varieties of *Phoenix dactylifera* were tested for antibacterial action against selected Gram positive and Gram negative pathogenic bacteria. Barring *Enterococcus faecalis*, the acetone and methanol extracts showed good antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Shigella flexeneri, Staphylococcus aureus,* and *Streptococcus pyogenes,* whereas the water extract had very little effect on all test bacterial species and there was almost negligible effect on *P. aeruginosa*. Pits extracts of all three varieties of *P. dactylifera* were found to be more effective than leaves extracts. Results clearly showed that *S. pyogenes* was most sensitive pathogen to the crude extracts and had shown maximum zone of inhibition. Minimum inhibitory concentration (MIC) for *S. pyogenes* was found to be 1.3, 1.1, 1.6 and 1.4 mg/ml for methanol leaves and pits extracts and acetone leaves and pits extracts, respectively.

Key words: Date palm, *Phoenix dactylifera* L, antibacterial, crude extracts.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a major fruit tree in most of Arabian Peninsula 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. Through international bibliography, three main cultivar groups of *P. dactylifera* have been described mainly according to sugar content of their fruit: (1) soft type (for example, 'Barhee'), (2) semi-dry type (for example, 'Dayri') and (3) dry type (for example, Thoory'). The beneficial health and nutrition values of date palm, for human and animal consumption, have been claimed for centuries (Duke, 1992; Vayalil, 2002; Tahraoui et al., 2007).

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 (Seelig, 1974; Dowson, 1982; Biglari et al., 2008). Four free phenolic acids and nine bound phenolic acids have been tentatively identified (Ziouti, 1996; Eong, 2006).

The number of trees in the Kingdom of Saudi Arabia is estimated to be over 23.5 million (Anonymous, 2009). These trees are estimated to yield about 210,000 tons of fronds (Al Gassim, 2011). Every year about three million palm trees are pruned and the portion becomes a waste. Similarly, pits are thrown after consuming pulp of date fruits, and thus these parts are considered as disposed waste. This attracted us for its use as a research material for analyzing antibacterial activity, initially as a control project. Al-Shahib and Marshall (2003) suggested the potential uses of dates seeds as sources of edible oils and pharmaceuticals.

During the last three decades the problem of antimicrobial resistance was getting increasingly acute and an increase in number of multi-drug 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

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in number of drug resistant bacteria is no longer matched by discoveries of new drugs to treat infections (Whitman, 2008).

According to World Health Organization, medicinal plants can be a good source of variety of drugs. Various societies across the world have shown great interest in curing diseases using plants/ plant based drugs. Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. 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 (Alagesaboopathi, 2011).

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 undertaken to evaluate the antibacterial properties of some cultivars of date palm of Saudi Arabia, 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 and dates of three cultivars that is, Barhee, Sukri and Rothana were evaluated for antibacterial activity. Fresh samples were kindly provided by Mr. Abdulmohssin Al-Shamlan from his Al-Shamlan date farm (Al-Qassim) Onaizah. Samples were labelled and stored at 4°C in polythene bags till they were processed.

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 15 days. Pits were manually removed from the fruits and samples were washed with distilled water and were dried at 40°C for 2 days in an oven (Memmert, Germany). The dried leaves and pit samples were ground well into a fine powder with the help of mill (IKA werke, GMBH & Co., Germany) and a mixer grinder (Philips, Brazil) respectively. The powder was stored in air sealed plastic containers at room temperature till extraction was carried out.

Extraction

The method of Alade and Irobi (1993) 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, acetone and methanol 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 Whatman No. 1 filter paper (Whatman, England). 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 strains *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Shigella flexeneri* (clinical isolate), *Staphylococcus aureus* ATCC 25923 and *Streptococcus pyogenes* (clinical isolate) were obtained from the Department of Microbiology, King Saud University, Riyadh, Saudi Arabia. Strains of bacteria were maintained on nutrient agar media (Merk, Germany).

Antibacterial assay

The crude extracts were screened against various human pathogens by agar well diffusion (Khan et al., 2011). In this method, 10 ml aliquots of nutrients broth (Sigma-Aldrich, Germany) 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 agar plate to obtain uniform inoculums. Four wells per plate were made with the reverse side of the sterilized micropipette tips. Crude extract (50 µl) was poured in respective wells with the help of micropipette. Doxycyline was used as positive control. Respective solvents were used as the negative control. Each extract was analyzed in triplicate. 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.

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was determined by the streak method (Hancock, 1997). To determine the MIC, extracts were dissolved in distilled water and serially diluted in eppendorf tubes under a laminar flow cabinet. The same volume of an actively growing culture of the tested pathogen was added to the different eppendorf tubes and cultures were grown overnight in an incubator at 37°C. The following morning, streaking was done from all samples on nutrient agar plates. MIC was rated by the lowest concentration of the test solution that inhibited growth.

RESULTS

In the present investigation, the antimicrobial activity of crude extracts of leaves and pits of three varieties of *P. dactylifera* was recorded against seven microbial species. With the exception of *E. faecalis*, the acetone and methanol extracts had shown good antibacterial activity against *B. subtilis, E. coli, P. aeruginosa, S. flexeneri, S. aureus,* and *S. pyogenes,* whereas water extract had very little effect on all test bacterial species and there was almost negligible effect on *P. aeruginosa*. Pit extracts of all three varieties were found to be more effective than leaves extract (Tables 1 to 3) and data on zone of inhibition indicates that these extracts were able to check the growth of all Gram negative bacteria as well as Gram positive bacteria except *E. faecalis,* which had not shown any zone of inhibition against any plant extracts used in the study.

Table 1 presents the antimicrobial activity of pits and leaves extracts of *P. dactylifera* vr. Barhee against *B. subtilis, E. coli, E. faecalis, P. aeruginosa, S. flexeneri*, *S. aureus*, and *S. pyogenes*. Data showed that after

Table 1. Antibacterial activity of crude extract of *Phoenix dactylifera* L. var. Barhee.

Bacteria	Inhibition zone (mm)[a]						
	Dc	Leaves			Pits		
		W	M	A	W	M	A
E. coli	20.3	5.7	18.3	17.7	5.3	21.0	18.7
S. aureus	24.3	8.3	16.7	16.3	6.3	21.0	18.3
B. subtilis	22.3	7.0	21.3	19.3	6.3	23.7	21.7
S. pyogenes	38.7	9.7	22.7	22.0	8.3	32.0	30.3
P. aeruginosa	23.0	-	20.0	19.3	-	19.7	19.0
E. faecalis	8.0	-	-	-	-	-	-
S. flexeneri	23.0	6.3	18.3	18.3	6.3	19.3	18.3

W, M, A, = Water, methanol and acetone extracts respectively. Standard: Dc = Doxycyline (10 mg). - = Not active against tested microorganism. [a]Data are the average of three experiments.

Table 2. Antibacterial activity of crude extract of *Phoenix dactylifera* L. var. Sukri.

Bacteria	Inhibition zone (mm)[a]						
	Dc	Leaves			Pits		
		W	M	A	W	M	A
E. coli	20.3	5.7	17.3	17.7	6.3	19.3	17.3
S. aureus	24.3	8.7	18.3	18.0	6.0	20.3	17.0
B. subtilis	22.3	7.0	20.3	18.0	-	21.0	19.7
S. pyogenes	38.7	9.3	20.0	20.3	6.7	33.0	31.3
P. aeruginosa	23.0	-	19.7	18.7	-	17.7	16.7
E. faecalis	8.0	-	-	-	-	-	-
S. flexeneri	23.0	6.3	17.0	19.3	7.0	17.7	16.7

W, M, A, = Water, methanol and acetone extracts respectively. Standard: Dc = Doxycyline (10 mg). - = Not active against tested microorganism. [a]Data are the average of three experiments.

doxycyline (standard positive control), methanol extracts of pits was most effective against all test bacterial strains except *E. faecalis* followed by acetone pits extract, methanol leaves extract, acetone leaves extract and aqueous extracts. Results clearly showed that *S. pyogenes* was most sensitive pathogen to the plant extract, whereas *P. aeruginosa* was found to be resistant to water extract of leaves and pits.

Data in Table 2 represents the antimicrobial activity of pits and leaves extracts of *P. dactylifera* vr. Sukri against *B. subtilis, E. coli, E. faecalis, P. aeruginosa, S. flexeneri* (clinical isolate), *S. aureus,* and *S. pyogenes*. Methanol pits extract showed maximum zone of inhibition against *S. pyogenes* (32.0 mm) followed by *B. subtilis, S. aureus, E. coli, P. aeruginosa* and *S. flexeneri* (23.7, 21.0, 21.0, 19.7, 19.3 mm respectively).

Similar results were obtained with pits and leaves extracts of *P. dactylifera* vr. Rothana (Table 3). Maximum zone of inhibition was observed with methanol pits extract against *S. pyogenes* (29.3 mm) followed by *B. subtilis, S. aureus, E. coli, P. aeruginosa* and *S. flexeneri* (23.7, 21.0, 20.7, 19.7, 19.3 mm, respectively).

Data generated from the present study revealed that all three varieties of date palm showed more or less similar antibacterial activity against all pathogenic bacteria tested in the study; therefore MIC was determined only for one variety of date palm. To determine MIC different concentration of methanol and acetone extracts of leaves and pits were used against the most sensitive bacteria that is, *S. pyogenes*. MIC for *S. pyogenes* was found to be 1.33, 1.15, 1.6 and 1.4 mg/ml for methanol leaves extract, methanol pits extract, acetone leaves extract and acetone pits extract respectively.

DISCUSSION

Numerous plants and secondary metabolites isolated from plants have been reported to possess antimicrobial properties (Ali and Qasir, 2009; Qadrie et al., 2009; Nisar et al., 2010; Khan et al., 2011; Samiullah et al., 2011).

Results of the present study showed that crude extract of leaves and pits checked the growth of all Gram negative and Gram positive bacteria except *E. faecalis*

Table 3. Antibacterial activity of crude extract of *Phoenix dactylifera* L. var. Rothana.

Bacteria	Inhibition zone (mm)[a]						
	Dc	Leaves			Pits		
		W	M	A	W	M	A
E. coli	20.3	5.7	17.0	16.3	7.0	20.7	18.3
S. aureus	24.3	5.7	15.7	17.7	6.0	21.0	20.0
B. subtilis	22.3	8.7	20.7	19.7	6.7	23.7	23.3
S. pyogenes	38.7	7.0	20.3	20.0	7.0	29.3	29.3
P. aeruginosa	23.0	-	19.7	18.7	-	19.7	14.3
E. faecalis	8.0	-	-	-	-	-	-
S. flexeneri	23.0	9.3	17.7	21.0	6.0	19.3	17.0

W, M, A, = Water, methanol and acetone extracts respectively. Standard: Dc = Doxycyline (10 mg). - = Not active against tested microorganism. [a]Data are the average of three experiments.

(Tables 1 to 3). Earlier, it has been reported that methanol and acetone extracts of the *P. dactylifera* pits moderately inhibited the growth of Gram positive and Gram negative bacteria (Sabah et al., 2007; Ammar et al., 2009).

Furthermore, the leaves and pits extracts have shown promising antibacterial activity against *S. pyogenes.* In an earlier study it was shown that *P. dactylifera* fruit extract neutralised the hemolytic activity of the streptococcal exotoxin, streptolysin O and 96% inhibition was obtained at a very low concentration (1:262144 DE dilutions) (Nizar et al., 1999).

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 (Cragg et al., 1996). The inhibition potential of plant extracts against the growth of microbes was attributed to the presence of antioxidants (Cutter, 2000; Puupponen et al., 2001). 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 (Dowson, 1982; Mosa et al., 1986; Ziouti, 1996; Eong, 2006; Biglari et al., 2008).

The results of our study demonstrated excellent antimicrobial activity by date palm 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. 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.

Conclusions

Relying upon the results obtained from this study it may be concluded that active antimicrobial compounds isolated from different extracts of *P. dactylifera* leaves and pit could be useful in treating diseases caused by the bacteria under study. However, an extensive study would be needed to extrapolate laboratory results into hospital settings for the benefit of mankind.

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