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Full Length Research Paper

In vitro inhibition potential of *Phoenix dactylifera* L. extracts on the growth of pathogenic fungi

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Antifungal potential of water, acetone and methanol extracts of leaves and pits of *Phoenix dactylifera* L. var. Barhee and Rothana were evaluated against seven pathogenic fungi. For assessing the antifungal activity, agar well diffusion and agar dilution methods were employed. Barring the water extracts, acetone and methanol extracts showed varying degree of growth inhibition of *Fusarium oxysporum, Fusarium sp., Fusarium solani, Aspergillus flavus, Alternaria alternata, Alternaria* sp. and *Trichoderma* sp. Methanol pits extract was highly effective against most of the tested fungi followed by methanol leaves extract, acetone pits and acetone leaves extract. The most sensitive fungus was *A. alternata*, its growth inhibition was maximum (51.6%) by methanol pits extract of var. Barhee. 50% inhibitory effect (IC_{50}) of methanol pits extract of var. Barhee against *F. oxysporum, F. solani, Fusarium sp., A. alternata* and *Alternaria* sp. was found to be 6.7, 4.9, 3.4, 2.8 and 3.7 mg/ml, respectively.

Key words: Antifungal potential, crude extracts, date palm, *Phoenix dactylifera*, pathogenic fungi.

INTRODUCTION

Phoenix dactylifera L. is a monocotyledonous woody perennial belonging to the Arecaceae family, which comprises 200 genera and 3000 species (Hickman, 1993). Date palm is a major fruit tree in most of Arabian Peninsula and it is considered to be one of the most important commercial crops. The beneficial health and nutrition values of date palm, for human and animal consumption have been claimed for centuries (Duke and Boca, 2001; Vayalil, 2002; Tahraoui et al., 2007). Several studies on antimicrobial activity of *P. dactylifera* have been reported (Vyawahare et al., 2009); however antifungal potential of date palm extracts have not been explored. Fungi are very large and diverse group of organisms, which are responsible for causing large number of diseases to human beings, animals and plants (Mehrotra, 1993).

Species of *Fusarium, Aspergillus, Alternaria* and *Trichoderma* are responsible for plant diseases such as blight, fruit rot, root crown rot, dieback and wilt (Fu and Chang, 1999; Demirci and Maden, 2006; Perveen and Bokhari, 2010). Apart from causing diseases in plants, many of these species are also a source of important mycotoxins to animals and humans (Placinta et al., 1999). Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* may cause liver cancer. Mycotic infectious rate increased significantly in association with several changes in medical practices, more over there have been increasing reports of antifungal resistance in humans (Joseph et al., 2003). Concerns have been raised about both the environmental impact and the potential health risk related to the use of synthetic compounds.

This led to intensify the search for new, safer, and more efficacious agents to combat serious fungal infections. One approach is the testing of plants traditionally used for their antifungal activities as potential sources for drug/fungicide development. The numbers of date palm in the Kingdom of Saudi Arabia are 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. Thus, this

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attracted us to utilize it as a research material for analyzing the antifungal activity, initially *in vitro* conditions.

MATERIALS AND METHODS

Collection and storage of plant samples

Fresh leaves and dates of two varieties that is, Barhee and Rothana were evaluated for antifungal activity. Fresh samples were kindly provided by Mr. Abdulmohssin Al-Shamlan from his Al-Shamlan date farm, Onaizah (Al-Qassim). 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 and 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 gm 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 antifungal activity was determined.

Test organisms

Pure cultures of fungal strains, *F. oxysporum, Fusarium sp., F. solani, A. flavus, A. alternata, Alternaria* sp. and *Trichoderma* sp. were used in the present study. Stock cultures of test fungi were maintained on potato dextrose agar (PDA, Scharlau Chemie, Spain) slants and were stored at 4 °C.

Determination of inhibition potential of the plant extract against pathogenic fungi

The water, acetone and methanol extracts of leaves and pits of *P. dactylifera* var. Barhee and Rothana were evaluated against pathogenic fungi by agar well diffusion and agar dilution method.

Antifungal assay by well diffusion

Antifungal assay of water, acetone and methanol extracts of leaves and pits of two varieties of *P. dactylifera* were evaluated against pathogenic fungi by measuring the diameter of the inhibition zone formed around the well. Test fungal suspension prepared in sterilized distilled water (1.0 × 10^6 spores/ml) was spread on PDA with the help of sterilized cotton swab. The extracts (50 µl/well) were placed in wells made on the pathogen inoculated agar plates. Respective solvents were used as control. Plates were incubated for 3 days at 26 ± 2°C, and inhibition zones of mycelial growth around the wells were measured. Each extract was analyzed in triplicate.

Antifungal assay by agar dilution method

On the basis of well diffusion results, the plant extracts and pathogenic fungi were further selected for antifungal evaluation by agar dilution method. Acetone and methanol extracts of leaves and pits of two varieties of *P. dactylifera* were added into 15 ml PDA. Mycelial plugs (5 mm in diameter) from the edges of each culture were incubated in the center of each PDA plate (90 mm diameter). Cultures were incubated at 26 ± 2°C for 7 days. Tests were repeated in triplicate. Percent inhibition was calculated as follows:

$$\text{Percent inhibition} = (DC- DT)/DCx100$$

Where DC: the diameter of growth zone in the control plate, DT: the diameter of growth zone in the test plate. IC_{50} (concentration that produces a 50% inhibitory effect) value of most effective extract against pathogenic fungi was graphically obtained from the dose response curves based on measurement at five different concentrations as reported earlier by Chang et al. (2008).

Statistical analysis

All experiments were performed in triplicate. The data were analyzed by least significant difference (LSD) test at probability of 0.05 to identify the significant effect of the treatment. Duncan Multiple Range Test was carried out to evaluate the significant differences between treatments ($P \leq 0.05$). ANOVA analysis was done with the SPSS statistics software.

RESULTS

In the present investigation, the antifungal activity of crude extracts of leaves and pits of two varieties of *P. dactylifera* were recorded against *F. oxysporum, Fusarium sp., F. solani, A. alternata, Alternaria* sp., *A. flavus* and *Trichoderma* sp. Results of well diffusion method presented in Table 1 shows that the acetone and methanol extracts had good inhibition potential against *F. oxysporum, Fusarium sp., F. solani, A. alternata and Alternaria* sp. Though acetone leaves extracts of *P. dactylifera* var. Barhee and Rothana showed poor inhibition against *Fusarium sp.* and *Alternaria* sp., respectively. Whereas, least zone of inhibition was observed against *A. flavus* and *Trichoderma* sp. Water extract had almost negligible effect on all tested fungi. Agar dilution method was used to represent the percent growth inhibition of *F. oxysporum, Fusarium sp., F. solani, A. alternata* and *Alternaria* sp. by pits and leaves extracts of *P. dactylifera* var. Barhee (Figure 1). Data showed that methanol and acetone extracts of pits and leaves were able to inhibit significantly the growth of all tested fungi ($P \leq 0.05$).

Methanol extracts of pits was highly effective against most of the fungi followed by methanol leaves extract, acetone pits and acetone leaves extract. *A. alternata*

Table 1. Evaluation of antifungal activity of crude extracts of *P. dactylifera* var. Barhee and Rothana by well diffusion method.

Fungi	Inhibition zone[a]											
	Leaves extract						Pits extract					
	Barhee			Rothana			Barhee			Rothana		
	w	A	m	w	a	m	w	a	m	w	a	m
A. alternata	-	+	++	-	+	+	-	+	+	-	+	++
A. flavus	-	-	±	-	-	±	-	-	±	-	-	±
Alternaria sp.	-	+	+	-	±	+	-	-	+	-	+	+
F. oxysporum	-	+	+	-	+	+	-	+	+	-	+	+
Fusarium sp.	-	±	+	-	+	+	-		+	-	+	+
F. solani	-	+	+	-	+	+	-	+	+	-	+	+
Trichoderma sp.	-	±	±	-	±	±	-	±	±	-	±	±

w, a, m, = water, acetone and methanol acetone extracts respectively. - = Not active against tested fungi, ± = poor antifungal activity, + = good antifungal activity, ++ = strong antifungal activity. [a]Experiment was carried out in triplicates.

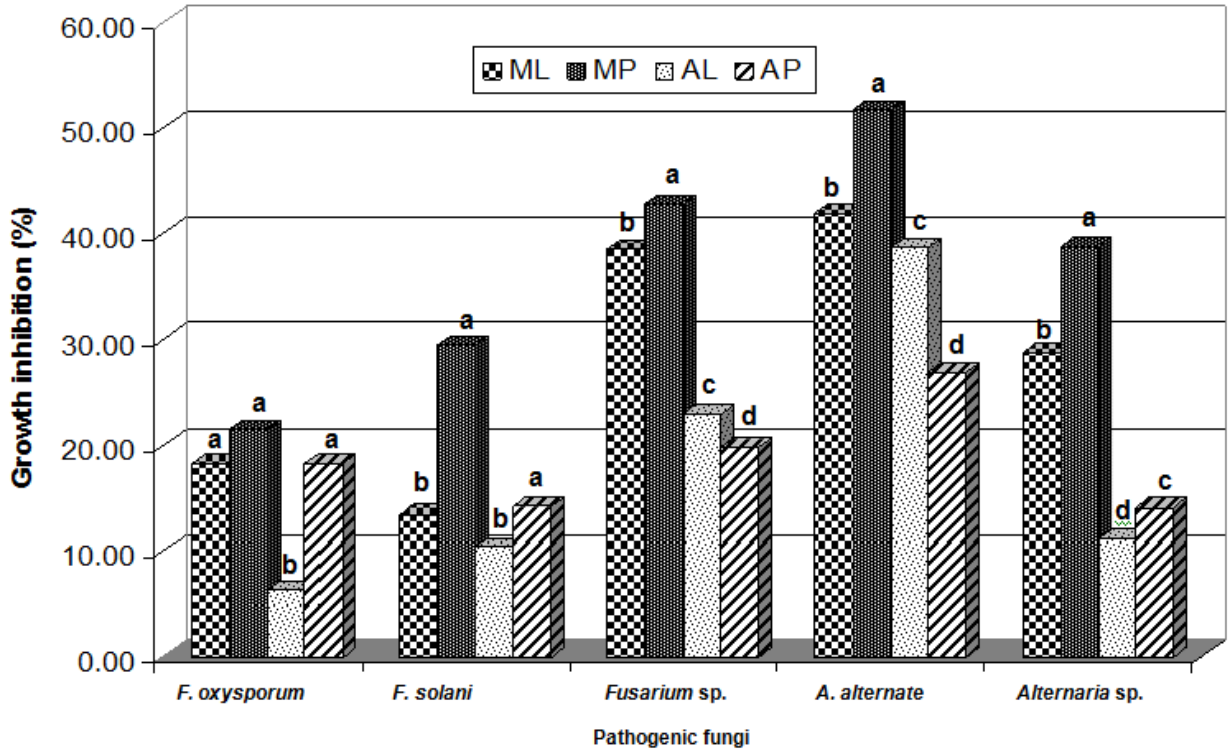

Figure 1. Percent growth inhibition of pathogenic fungi by methanol and acetone extracts of *P. dactylifera* var. Barhee (30 µl/ml) by agar dilution method. Each value is an average of three replicates. Bars with different letters are significantly different (P ≤ 0.05) according to Duncan's multiple range test. ML = Methanol leaves extract, MP = Methanol pits extract, AL = Acetone leaves extract, AP = Acetone pits extract.

showed maximum growth inhibition by methanol pit extracts (51.6%) whereas the reduction in *F. solani* radial growth due to methanol pit extract was 29.4%. However, microscopic observations showed that spore count was comparatively low in methanol pits extract diluted agar plate than respective control plate. Acetone leaves extract showed least growth inhibition of *F. oxysporum* (6.3%). The antifungal assay of methanol and acetone extracts of *P. dactylifera* var. Rothana by agar dilution method is presented in Figure 2. Results indicated that all extract significantly inhibited the radial growth of fungi (P ≤ 0.05). The methanol pits extract was most effective in inhibiting growth of *A. alternata* (40.9%), whereas growth of *Fusarium* sp. was equally inhibited by both methanol

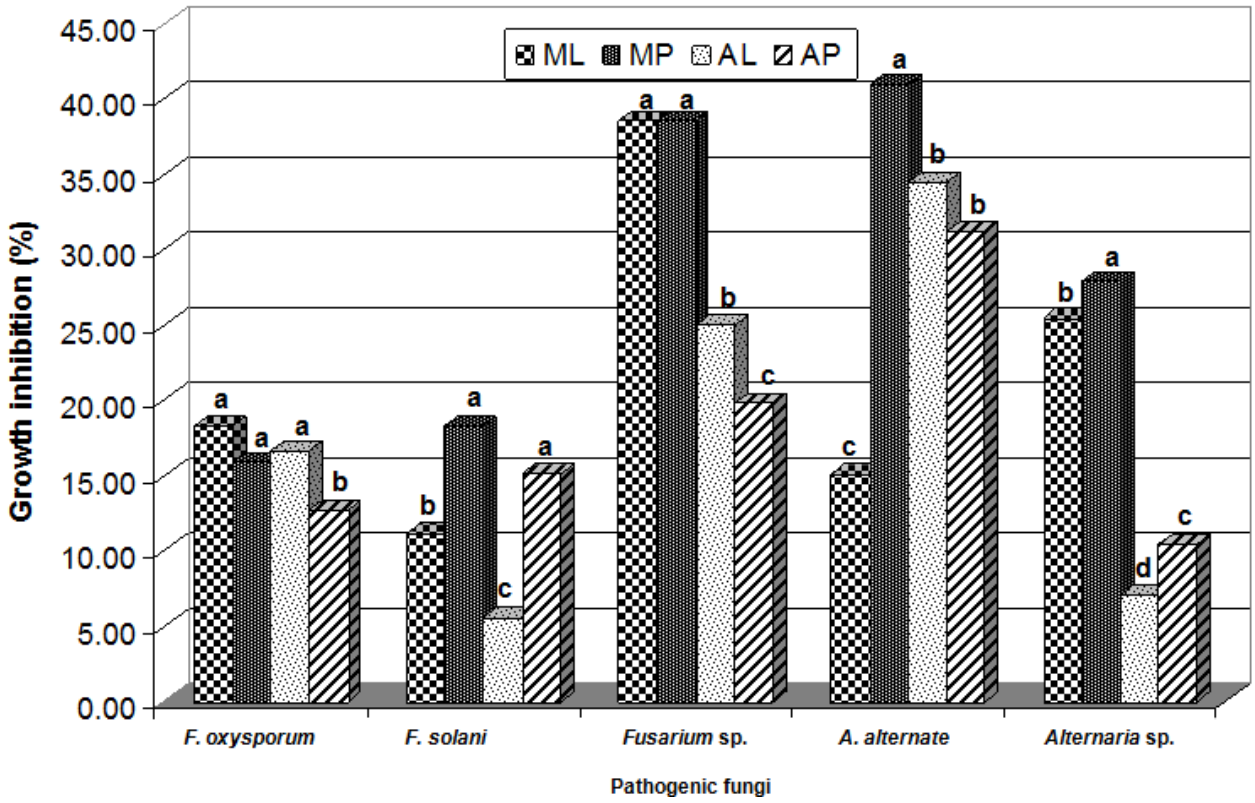

Figure 2. Percent growth inhibition of pathogenic fungi by methanol and acetone extracts of *P. dactylifera* var. Rothana (30 µl/ml) by agar dilution method. Each value is an average of three replicates. Bars with different letters are significantly different (P ≤ 0.05) according to Duncan's multiple range test. ML = Methanol leaves extract, MP = Methanol pits extract, AL = Acetone leaves extract, AP = Acetone pits extract.

pits and leaves extracts (38.5%). Acetone leaves extract had least effect on the radial growth of *F. solani* (5.6 %). Data generated from the present study revealed that methanol pits extract *of P. dactylifera* var. Barhee was most effective against all pathogenic fungi tested. Therefore IC_{50} was determined only for methanol pits extract of *P. dactylifera* var Barhee. IC_{50} for methanol pits extract against *F. oxysporum, F. solani, Fusarium sp., A. alternata* and *Alternaria* sp. was found to be 6.7, 4.9, 3.4, 2.8 and 3.7 mg/ml, respectively.

DISCUSSION

In the present study, methanol and acetone extracts of leaves and pits of two varieties of *P. dactylifera* inhibited the growth of *F. oxysporum, Fusarium sp., F. solani, A. alternata, Alternaria* sp. Water extract had almost negligible effect on all fungi tested (Table 1). 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). However reports on antifungal activity of *P. dactylifera* are unavailable. Different concentrations of *Spilanthes acmella* showed high inhibition zones in *F. oxysporum* and *F. moniliformis* followed by *Aspergillus niger* and *A. paraciticus* (Sabitha et al., 2006). In the present study, negative results may be due to insufficient quantities of active compound(s) in the crude extracts to show activity with the dose levels employed. Data presented in Figures 1 to 2 showed that methanol extracts of pits was most effective against all tested pathogenic fungi followed by methanol leaves extract, acetone pits extract and acetone leaves extract.

Results clearly showed that *A. alternata* was most sensitive fungus to the plant extract. Latif et al (2006) found that plant extracts prepared from garlic, neem leaf, ginger and onion bulb were able to control *Alternaria, Aspergillus, Chaetomium*, *Curvularia, Fusarium, Penicillium* and *Rhizopus*. Similarly, Jabeen et al. (2008) found that seed extracts of *Moringa oleifera* inhibited the growth of *F. solani* and *Rhizoctonia solani*. Earlier various studies reported that plant extracts had a good potential to control various fungal diseases (Abd-El-Khair et al., 2007; Joseph et al., 2008; Yasmin et al., 2008; Mdee et al., 2009; Yanar et al., 2011; Salim, 2011). The phytochemicals derived from root, stem, leaves, fruits, flowers and seeds of medicinal plants include phenolic

compounds, essential oils, proteins and antioxidants, and 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 positive antifungal activity by date palm extracts against tested pathogenic fungi, which might be due to the selective or synergistic action of various chemicals present in date palm. There were some differences in the antifungal activities of extracts which may be due to differences in phytochemicals found in two varieties of *P. dactylifera*.

Conclusions

On the basis of 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 controlling pathogenic fungi. However, to know the exact mechanism of action of *P. dactylifera* extracts, further studies with purified fractions/bioactive compounds are recommended.

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