J. Agric. Chem. and Biotechn., Mansoura Univ. Vol. 3 (8): 277 - 284, 2012

IN VITRO ANTIFUNGAL ACTIVITY OF THREE SAUDI PLANT EXTRACTS AGAINST SOME PHYTOPATHOGENIC FUNGI

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ABSTRACT

The antifungal activities of ethanolic extracts of three Saudi plants namely, camel thorn (*Alhagi maurorum* Medic.), caper (*Capparis spinosa* L.) and pomegranate (*Punica granatum* L.) were investigated *in vitro* against *Alternaria alternata, Fusarium oxysporum, Phoma destructiva, Rhizoctonia solani* and *Sclerotium rolfsii* at concentrations of 0, 3, 6, and 9% (v/v). All tested plant extracts (seeds, roots and rinds) had an antifungal activity at different degrees against the tested fungi. The highest antifungal activity was recorded for camel thorn seeds extract at concentration of 9% when compared with the control, while, pomegranate rinds extract at 9% came at the second rank. On the other hand, camel thorn rinds extract came at the end even at high concentration. The ethanolic extract of camel thorn seeds may be recommended as a potent bio-fungicide. Extensive studies should be undertaken for the ethanolic extract of camel thorn seeds as a strong antifungal agent against fungal plant diseases.

Keywords:*Alternaria alternata, Fusarium oxysporum, Phoma destructiva, Rhizoctonia solani* and *Sclerotium rolfsii.*

INTRODUCTION

Fungal infections cause significant loss in many economic crops. Crop losses are estimated to be about 14% worldwide (Agrios, 2005). Among the phytopathogenic fungi, *Alternaria alternata* (Fr.) Keissl, *Fusarium oxysporum* Schlecht., *Phoma destructiva* Plowr., *Rhizoctonia solani* Kühn. and *Sclerotium rolfsii* Sacc. are reported as destructive ones. They cause leaf spots, *Fusarium* wilt, Phoma rot, *Rhizoctonia* root rot and root and stem-rot on a wide variety of agricultural crops, respectively (Yaqub and Shahzad, 2005; Abdel-Fattah *et al.*, 2011 and Alwathnani and Perveen, 2012).

Chemical control may be available to reduce the effects of most fungal disease effectively and extensively, but field application of these chemical fungicides may not always be desirable. Excessive and improper use of these fungicides presents a menace to the health of humans, animals and environment. Therefore, considerable search for biofugicides that are environmentally safe and easily biodegradable have been carried out during last two decades (Gnanamanickam, 2002).

Investigation of plants containing natural antimicrobial metabolites for plant protection has been identified as a desirable method of disease control (Rai and Carpinella, 2006). Various plant products like plant extracts, essential oils, gum, resins... etc. were shown to exert biological activity *in vitro* and *in vivo* and are used as bio-fungicidal compounds (Fawzi *et al.* 2009 and Al-Askar and Rashad 2010). The main reasons for using essential oils as antifungal agents are their natural origin and low chance of pathogens

developing resistance. They may have a minimum adverse effect on physiological processes of plants and less environmental hazards compared to their synthetic alternatives, being plant products are easily convertible into a common organic material (eco-friend) (Gnanamanickam, 2002).

This work was aimed to investigate the antifungal activity of ethanolic extracts of camel thorn (*Alhagi maurorum* Medic.), caper (*Capparis spinosa* L.) and pomegranate (*Punica granatum* L.) *in vitro* on growth of the tested fungi (*A. alternata*, *F. oxysporum*, *P. destructiva*, *R. solani* and *S. rolfsii*).

MATERIALS AND METHODS

Plant material and fungi

Three Saudi plant species (camel thorn, caper and pomegranate) were collected from the various parts of Riyadh region in Saudi Arabia. Plants were randomly collected to increase the chance of finding plants with bioactive extracts. The plants were identified by the Herbarium at King Saud University, College of Food and Agricultural Sciences.

The fungal strains *A. alternata*, *F. oxysporum*, *P. destructiva*, *R. solani* and *S. rolfsii* were isolated originally from different naturally diseased plants collected from different agricultural fields in Riyadh region. All fungi were cultured on potato dextrose agar (PDA) (Difco, USA) plates and incubated at 28°C for one week. Purification of the resulting isolates was done using the hyphal tip or single spore techniques to obtain pure cultures; the detected isolates were then transferred into slant of PDA and kept at 4° C for further studies. Pure cultures of the isolated fungi were identified according to the cultural properties, morphological and microscopical characteristics of each fungus (Domsch *et al.*, 1980; Burgess and Liddell, 1983 and Watanabe, 2002).

In vitro antifungal activity of the plant extracts

The plant materials seeds and rinds of camel thorn, caper roots and pomegranate rinds were washed with distilled water and dried in shade. They were then finely grinded to powder. Fifty grams of each plant material in powder form was homogenized by laboratory blender in 200 ml of ethanol (96%) and distilled water (20: 80 v/v) for 10 min, and then left in dark glass bottles for 72 h at room temperature for complete extraction. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60°C in water bath for 30 min for ethanol evaporation. The collected extracts were then stored in a refrigerator at 5°C until needed. The plant extracts were added to conical flasks containing sterilized PDA before solidification to obtain the proposed concentrations of 0, 3, 6, and 9% (v/v). 20 ml of amended media were poured into 9 cm diameter Petri dishes. For each treatment, 3 replicates (plates) were used. All plates were inoculated individually with 0.5 cm diameter discs of the tested fungal cultures, and then incubated in the dark at 28±2°C, the linear growth of each fungus was measured 3, 6 and 9 days after inoculation. All plates were arranged in complete randomized design.

Statistical Analysis

All data were subjected to analysis of variance using the statistical analysis software (CoStat, 2005). Comparisons among means were made using Duncan's multiple range test (Duncan, 1955).

RESULTS

***In vitro* antifungal activity of the plant extracts**

The antifungal activities of the plant extracts (seeds and rinds of camel thorn, caper roots and pomegranate rinds) were investigated against the linear growth of five fungi at different concentrations (3, 6 and 9 %). Data of growth reduction of *R. solani* in response to the tested plant extracts is presented in Table 1. All tested plant extracts had an antifungal activity at different degrees against *R. solani*. Three days after inoculation, it was found that there is an inverse relationship between the concentration of the plant extracts and the linear growth of *R. solani i.e.* as the concentration increases the linear growth decreases. The highest antifungal activity was recorded for camel thorn seeds extract at concentration of 9% when compared with all other treatments. The linear growth of *R. solani* continued to increase after 6 days of inoculation at different concentrations of the tested plant extracts. At day 9, plates treated with the plant extracts (camel thorn rinds, caper roots or pomegranate rinds) at 3 and 6% reached the full growth. Camel thorn seeds extract at 9% concentration continued to be the most effective inhibitor for the growth of *R. solani* compared with the control after 9 days of inoculation. In this connection, camel thorn rinds extract came at the end even at high concentration, while caper roots came at the second rank and pomegranate rinds extracts at the third rank.

Results presented in Table 2 show the effects of the tested plant extracts on the linear growth of *F. oxysporum*. At day 3, plant extracts of camel thorn seeds, caper roots or pomegranate rinds significantly reduced the linear growth of *F. oxysporum* at different degrees with the increase of the concentrations. In the same time, it was found that camel thorn rinds extract significantly reduced the linear growth of *F. oxysporum* but the inhibitory effect significantly decreased with the increase in the concentration. The linear growth of *F. oxysporum* continued to increase after 6 days of inoculation at different concentrations of the tested plant extracts. At day 9, camel thorn seeds extract at 9% concentration continued to be the most effective inhibitor for the growth of *F. oxysporum* compared with the control, while, pomegranate rinds extract came at the second rank.

At day 3, it was observed that the inhibitory effect of the plant extracts against *S. rolfsii* significantly increased with the increase in the concentration except camel thorn rinds extract, (Table 3) which recorded the same observation with *F. oxysporum*. In this connection, the linear growth of *S. rolfsii* continued to increase after 6 days of inoculation at different concentrations of the tested plant extracts. The highest antifungal activity was recorded for camel thorn seeds extract at concentration of 9% when

compared with the control after 9 days of inoculation, while pomegranate rinds extracts came at the second rank and caper roots at the third rank.

Table 1: Effect of different plant extracts on the linear growth of *R. solani*.

Time	Inward linear growth* (cm)												
	Cont	Camel thorn seeds			Camel thorn rinds			Caper roots			Pomegranate rinds		
		3 %	6%	9%	3 %	6%	9%	3 %	6%	9%	3 %	6%	9%
days	9.0 a	3.9 h	2.1 j	1.1 k	9.0 a	7.0 b	6.8 c	5.4 e	4.4 g	2.9 i	6.4 d	4.4 g	3.0 i
days	9.0 a	4.4 e	2.4 f	1.1 g	9.0 a	9.0 a	9.0 a	8.8 b	8.0 c	4.4 e	9.0 a	9.0 a	5.4 d
days	9.0 a	4.9 d	2.4 e	1.1 f	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	5.2 c	9.0 a	9.0 a	6.9 b

* Each value represents the mean of 3 replicates.

** Values within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 2 : Effect of different plant extracts on the linear growth of *F. oxysporum*.

Time	Inward linear growth* (cm)												
	Cont	Camel thorn seeds			Camel thorn rinds			Caper roots			Pomegranate rinds		
		3 %	6%	9%	3 %	6%	9%	3 %	6%	9%	3 %	6%	9%
3 days	3.1 a	2.0 g	2.0 g	1.8 h	2.6 c	2.7 bc	2.8 b	2.5 d	2.4 de	2.3 ef	2.2 f	1.8 h	1.4 i
6 days	7.7 a	2.9 g	2.5 h	2.1 i	4.6 de	5.1 c	5.9 b	5.2 c	4.8 d	4.5 e	4.5 e	3.3 f	2.5 h
9 days	9.0 a	3.8 h	3.2 i	2.5 j	6.6 f	7.6 d	8.3 b	8.0 c	7.2 e	6.6 f	6.8 f	5.0 g	3.7 h

* Each value represents the mean of 3 replicates

** Values within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

In vitro antifungal activities of the plant extracts against *A. alternata* are presented in Table 4. At day 3, comparing with the control, it was observed that plant extracts of camel thorn seeds, caper roots or pomegranate rinds significantly reduced the linear growth of *A. alternata* with the increase in the concentrations. In contrast, no additional inhibitory effect of the plant extract of camel thorn rinds with the increase in its concentration. The linear growth of *A. alternata* continued to increase after 6 days of inoculation at different concentrations of the tested plant extracts. At day 9, the highest antifungal activity was recorded for camel thorn seeds extract at concentration of 9% when compared with the control, while caper roots and pomegranate rinds extracts came at the second rank. On the other hand, camel thorn rinds extract came at the end even at high concentrations.

Effect of different plant extracts on the linear growth of *P. destructiva* is presented in Table 5. At day 9, no significant differences were recorded in the linear growth of *P. destructiva* when treated with the plant extracts of camel thorn rinds, caper roots or pomegranate. In contrast, plant extract of camel thorn seeds significantly decreased the linear growth of *P. destructiva* with the increase in its concentration when compared with the control even after 9 days of inoculation.

DISCUSSION

The geographical location of Saudi Arabia has provided an ideal environment for the growth and nourishment of different medicinal plant species including camel thorn, caper and pomegranate. The country is gifted with diverse vegetation types occurring in the desert, semi-desert, and mountainous ecosystems (Ahmad and Ghazanfar, 1991).

Table 3: Effect of different plant extracts on the linear growth of *S. rolfsii*.

Time	Inward linear growth* (cm)												
	Cont	Camel thorn seeds			Camel thorn rinds			Caper roots			Pomegranate rinds		
		3 %	6%	9%	3 %	6%	9%	3 %	6%	9%	3 %	6%	9%
3 days	2.1 a	1.7 b	1.0 e	0.8 f	1.6 b	2.0 a	2.0 a	1.5 c	1.5 c	1.3 d	1.7 b	1.5 c	1.4 cd
6 days	4.0 a	1.4 g	1.2 h	0.8 i	2.4 de	4.0 a	4.0 a	2.7 c	2.5 d	2.4 e	2.8 b	2.3 e	2.2 f
9 days	6.1 a	1.5 f	1.4 f	0.9 g	3.2 e	5.7 b	5.8 b	3.9 c	3.6 d	3.3 e	3.8 d	3.2 e	3.3 e

* Each value represents the mean of 3 replicates

** Values within a row followed by the same letter(s) are not significantly different according to Duncan's Multiple range test ($p \leq 0.05$)

Table 4:Effect of different plant extracts on the linear growth of *A. alternata*.

Time	Inward linear growth* (cm)												
	Cont	Camel thorn seeds			Camel thorn rinds			Caper roots			Pomegranate rinds		
		3 %	6%	9%	3 %	6%	9%	3 %	6%	9%	3 %	6%	9%
3 days	2.9 a	1.7 d	1.5 e	0.8 f	2.6 b	2.8 a	2.9 a	2.2 d	1.7 d	1.5 e	2.2 c	1.6 e	1.5 e
6 days	6.9 a	2.3 i	2.0 j	1.1 k	5.2 c	6.7 b	6.7 b	3.5 e	3.3 f	2.8 g	4.9 d	3.6 e	2.7 h
9 days	9.0 a	2.8 i	2.5 j	1.3 k	7.1 c	9.0 a	9.0 a	5.1 e	4.6 f	4.1 h	7.4 b	5.3 d	4.4 g

* Each value represents the mean of 3 replicates

** Values within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

Table 5: Effect of different plant extracts on the linear growth of *P. destructiva*.

Time	Inward linear growth* (cm)												
	Cont	Camel thorn seeds			Camel thorn rinds			Caper roots			Pomegranate rinds		
		3 %	6%	9%	3 %	6%	9%	3 %	6%	9%	3 %	6%	9%
3 days	9.0 a	6.9 c	5.1 d	1.8 e	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	7.2 b
6 days	9.0 a	6.9 b	4.9 c	1.7 d	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a
9 days	9.0 a	7.2 b	5.1 c	1.7 d	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a

* Each value represents the mean of 3 replicates.

** Values within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

The antifungal activities of the tested plant extracts (camel thorn, caper and pomegranate) were investigated at different concentrations. Our results indicated that all tested plant extracts had an antifungal activity at different degrees against the tested fungi. The *in vitro* efficacy of pomegranate, caper and camel thorn against different pathogens has been investigated by various researchers (Lam *et al.*, 2009; Dahham *et al.*, 2010 and Abd-Ellatif *et al.*,

2011). The antifungal activity attained by these plant extracts is attributed to their chemical composition. Based on spectral analyses, the compound from pomegranate rinds extract that exhibit strong antifungal activity was previously identified as punicalagin. Punicalagin showed strong activity against *Candida* spp. (Endo *et al.*, 2010). Moreover, it has antibacterial, antioxidant, anticancer and anti-inflammatory properties (Miguel *et al.*, 2010). Previous chemical studies have reported that alkaloids, lipids, polyphenols, flavonoids, indole and glucosinolates were isolated from caper extract (Tlili *et al.*, 2010). The richness of caper plant with total phenolic compounds, rutin, tocopherols, carotenoids and vitamin C could be the main factor in its antimicrobial effects (Mahboubi *et al.*, 2012).

Our results revealed that the highest antifungal activity was recorded for camel thorn seeds extract at concentration of 9% when compared with the control. The obtained result is in accordance with that achieved by Abd-Ellatif *et al.*, (2011) on *Aspergillus flavus, A. alternata, F. oxysporum, F. solani, Bipolaris oryzae, Chetomium* sp. and *Mucor* sp. In another study, the ethanolic extract of camel thorn plant showed significant antimicrobial activity against Gram negative, Gram positive bacteria, unicellular and filamentous fungi (Zain *et al.*, 2012). Furthermore, it has some medicinal properties as antioxidant, anti-inflammatory (Awaad *et al.*, 2011), antiulcerogenic (Awaad *et al.*, 2006) and antidiarrhoeal activity (Gutierrez *et al.*, 2007). Phytochemical screening of camel thorn extract revealed the presence of flavonoids, glycosides, alkaloids, saponins, tannins, steroids and anthraquinone as major constituents (Abdel Rahman *et al.*, 2011). Additional constituents were reported in the camel thorn extract as β-sitosterol, cinnamic acid, coumaric acid, hydroxybenzoic acid (Ahmad *et al.*, 2009). The activity of camel thorn plant could be explained, at least by their antimicrobial properties, due to their high flavonoid contents.

The results of this study support the traditional usage of the studied plants. Hence, the objective of this study was to determine if plant extracts could provide antifungal activity against some phytopathogenic fungi. Considering their attribute and broad-spectrum activities, successful development of such compounds as antifungal would not only provide a potent tool for control of the tested pathogenic fungi, but also could promise success in multipurpose biorational alternatives to conventional fungicides for the management of other plant diseases. Extensive studies should be undertaken for the ethanolic extract of camel thorn seeds as a strong antifungal agent against fungal plant diseases.

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تأثير المضاد للفطريات معملياً لمستخلصات ثلاث نباتات سعودية ضد بعض الفطريات الممرضة للنبات

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تم اختبار التأثير المضاد للفطريات معملياً لمستخلصات كحولية (باستخدام الكحول الايثيلي) لثلاث نباتات صحراوية سعودية تشمل شوك الجمل، نبات العاقول ونبات الرمان ضد خمسة من الفطريات الممرضة للنبات و هي الترناريا الترناتا، فيوزاريوم اوكسيسبورم، فوما ديستركتيفا ، ريزوكتونيا سولاني و سكلوروتيم رولفسياي وذلك عند تركيزات 0، 3، 6 ، 9 %. وقد أظهرت النتائج ما يلي:

كل المستخلصات التي تم اختبارها أظهرت تضاداً فطرياً بدرجات مختلفة ضد الفطريات محل الدراسة. تم تسجيل أقوى تأثير تضادي لمستخلص بذور نبات شوك الجمل عند تركيز 9 % وذلك عند المقارنة مع الكنترول، بينما حل مستخلص قشر الرمان عند تركيز 9 % في المرتبة الثانية. من جهة أخرى حل مستخلص قشر نبات شوك الجمل أخيراً حتى عند أعلى تركيز مقارنة بالكنترول.

وتوصي الدراسة باستخدام مستخلص بذور نبات شوك الجمل كمضاد حيوي فطري مؤثر ، كما توصي كذلك بعمل دراسات أخرى تطبيقية وحقلية لهذا المستخلص ودراسة مدى امكانية تطبيقه عملياً كمضاد للفطريات المسببة للأمراض النباتية.

قام بتحكيم البحث

أ.د / فتحى اسماعيل على حوقه — كلية الزراعة – جامعة المنصورة

أ.د / وسام الدين اسماعيل على صابر — مركز البحوث الزراعية