

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; 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 different degrees of antifungal activity against the tested fungi. When compared with the control, the highest antifungal activity was recorded for camel thorn seeds extract at a concentration of 9%, while, pomegranate rinds extract at 9% came in second. Camel thorn rinds extract came in last even when used at a 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.

Key words: *Alternaria alternata*, *Fusarium oxysporum*, *Phoma destructiva*, *Rhizoctonia solani*, *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. 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; Alwathnani and Perveen 2012).

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

The 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, gums, resins *etc.* were shown to exert biological activity *in vitro* and *in vivo* and are used as bio-fungicidal compounds (Fawzi *et al.* 2009; Al-Askar and Rashad 2010). The main reasons for using essential oils as antifungal agents are because they are natural in

origin and because there is a low chance of pathogens developing resistance to them. Essential oils may have a minimum adverse effect on the physiological processes of plants and have less environmental hazards compared to their synthetic alternatives. Since essential oils are plant products, they are easily convertible into a common organic material (eco-friendly) (Gnanamanickam 2002).

The aim of this work was 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 the 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 the 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 originally isolated from different naturally diseased plants collected from different agricultural fields in the Riyadh region. All fungi were

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cultured on PDA (Potato Dextrose Agar) (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 a 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; Watanabe 2002).

In vitro antifungal activity of the plant extracts

The plant material, seeds, and rinds of camel thorn, caper roots, and pomegranate rinds were washed with distilled water and then dried in the shade. Next, they were finely ground to powder. Fifty grams of each plant material in powder form was homogenized in a 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 a 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). Twenty 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 a 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 concerning the growth reduction of *R. solani* in response to the tested plant extracts is presented in table 1. All tested plant extracts had different degrees of antifungal activity against *R. solani*. Three days after inoculation, it was found that there was 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 seed extract at a 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 full growth. Camel thorn seeds extract at a 9% concentration, continued to be the most effective inhibitor of *R. solani* growth, after 9 days of inoculation. Camel thorn rinds extract came in last even when used at high concentrations. Caper roots and pomegranate rind extracts ranked second.

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

Time	the control	Inward linear growth* [cm]											
		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**	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
6 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
9 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 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	the control	Inward linear growth* [cm]											
		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 ($p \leq 0.05$)

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

Time	the control	Inward linear growth* [cm]											
		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 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	the control	Inward linear growth* [cm]											
		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 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	the control	Inward linear growth* [cm]											
		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 are not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

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. At 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 a 9% concentration, continued to be the most effective inhibitor of the growth of *F. oxysporum* compared with the control, while, pomegranate rinds extract came in second.

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. Camel thorn rinds extract (Table 3) however, did not increase but the same observation with *F. oxysporum* was recorded. 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 seed extract at a concentration of 9% when compared with the control after 9 days of inoculation.

In vitro antifungal activities of the plant extracts against *A. alternata* are presented in table 4. At day 3, 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, there was no additional inhibitory effect with the increase of the plant extract from camel thorn rinds. The linear growth of *A. alternata* continued to increase after 6 days of inoculation with different concentrations of the tested plant extracts. At day 9, the highest antifungal activity was recorded for camel thorn seed extract at a concentration of 9% when compared with the control. On the other hand, camel thorn rinds extract, even at high concentrations, came in last.

The 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, camel thorn seeds extract 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).

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 different degrees of antifungal activity 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; 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 the pomegranate rinds extract that exhibited 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 the caper plant with the 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 a 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 the camel thorn plant showed significant antimicrobial activity against Gram negative, Gram positive bacteria as well as unicellular and filamentous fungi (Zain *et al.* 2012). Furthermore, it has some medicinal properties such 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). The additional constituents were reported in the camel thorn extract: β -sitosterol, cinnamic acid, coumaric acid, and hydroxybenzoic acid (Ahmad *et al.* 2009). The activity of camel thorn plants 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 antifungal compounds 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 about the ethanolic extract of camel thorn seeds as a strong antifungal agent against fungal plant diseases.

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