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Full Length Research Paper

In vitro antifungal activity of 63 Iranian plant species against three different plant pathogenic fungi

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Crude aqueous and methanolic extracts of 63 plant species belonging to 23 families collected from the west of Iran were screened for antifungal activity against three economically important phytopathogenic fungi, *Cochliobolus sativus*, *Fusarium oxysporum* and *Rhizoctonia solani.* Bioassay of extract was conducted by paper disc diffusion method on agar plate cultures with four replications. 21 of the 63 (33%) plant species showed inhibitory activity against at least one of the fungi. 16 (25%), 10 (16%) and 16 (25%) tested plant species inhibited the mycelial growth of *R. solani*, *F. oxysporum* and *C. sativus*, respectively. *Centaurea behen, Lavandula* sp., roots of *Tribulus terrestris* were the most active plant species against *R. solani*, *F. oxysporum*, and *C. sativus*, respectively. Extracts of *Glycyrrhiza glabra, Rosmarinus officinalis, Avena sativa, Vaccaria pyramidata, Centaurea behen, Anagalis arvensis* and *T. terrestris* exhibited a broad-spectrum of antifungal activity. According to these results, we conclude that the flora in the west of Iran can be regarded as a rich source of plants with antifungal activity. Therefore, further screening of other plant species, identifying active fractions or metabolites and *in vivo* application of active extracts are warranted.

Key words: Antifungal activity, crude extract, Iranian plants, paper disc, phytopathogenic fungi.

INTRODUCTION

Crop losses due to plant diseases are estimated to be about 14% worldwide (Agrios, 2005) and 20% for major foods and cash crops (Oerke et al., 1994). Synthetic pesticides are the most effective method of the pest and disease control. In spite of hazardous impacts of chemical pesticide application including problems of public health, environmental pollution, toxic effect on non-target organisms and causing resistance in pest and disease agents (Rai and Carpinella, 2006; Kagale et al., 2004), it is believed that fungicides will remain essential for the control of plant diseases and it should be optimized under integrated pest management programs (Gullino et al., 2000). Integrated pest management (IPM) for conserving agro-ecosystem include the use of pest-resistance cultivars, holding pests at tolerable levels and making use of natural products (Rai and Carpinella, 2006).

Regarding the problems created by synthetic pesticide application, environmentally safe methods are needed to replace chemical pesticides or reduce their consumption in ecosystem. Therefore, considerable search for biocides that are environmentally safe and easily biodegradable have been carried out during last two decades (Duke et al., 2003; Teggne et al., 2008). Investigation of plants containing natural antimicrobial metabolites for plant protection has been identified as a desirable method of disease control (Rai and Carpinella, 2006; Kim et al., 2002). Given the effect of the plant species origin and genetic diversity on chemical composition, studies screening for novel antifungal compounds in plants from different part of the world are

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needed. Hence, in this research, we screened plants from west of Iran.

Iran is divided to 31 provinces including Kermanshah and Hamadan, with a vast range of climatic conditions located in the west of the country. Plant diversity is very rich in these two provinces; therefore, it is expected to find significant and distinct variation in secondary metabolites with antifungal activity. Iranian plants have been screened previously for antimicrobial activity (Sardari et al., 1998; Fazly Bazzaz et al., 1997; Fazly Bazzaz and Haririzadah, 2003; Shahidi Bonjar et al., 2004), but with a focus on activity against agents of diseases in human. There have been no screening studies for activity of Iranian plants against the phytopathogenic fungi.

In this study, three destructive phytopathogenic fungi, *Cochliobolus sativus*, *Fusarium oxysporum* and *Rhizoctonia solani* were considered to test the antifungal activity of plant species. All the fungi are phytopathogenic fungi at farm level. Regarding the importance of screening plant crude extracts as first step of the project and the importance of bioactive crude extracts as eco-friendly agents, collected plants from the west of Iran were screened against all three fungi. The objective of the research was, as a part of larger screening program, to assess the antifungal activity of extracts from 63 randomly-collected plant species in Kermanshah and Hamadan.

MATERIALS AND METHODS

Plant material and fungi

63 plant species from 23 families were collected from the various parts of the provinces of Kermanshah and Hamadan in Western Iran (Table 1). As a part of a wider screening program, plants were randomly collected to increase the chance of finding plants with bioactive extracts. The plants were identified by the Herbarium at Razi University, College of Agriculture and the scientific names were checked in the International Plant Names Index (http://www.ipni.org/ipni/plantnamesearchpage.do). Each sample was cleaned, air dried in the shade and ground to a fine powder with a coffee grinder. More also, three economically important phytopathogenic fungi, *C. sativus* (S. Ito and Kurib.), *F. oxysporum* Schlecht. and *R. solani* Kühn were provided by the Agriculture and Natural Resources Research Centre of Kermanshah and the Plant Pathology Laboratory, College of Agriculture, Razi University.

Preparation of plant extracts

The powdered plant materials were extracted at room temperature using water and methanol. Aqueous extraction was achieved by adding 100 ml distilled water to 5 g of plant powder and brought to boil. Once boiled, the suspension was allowed to stand for 4 h before being filtered. The extract was then concentrated using a rotary evaporator. A sample of extract at concentration of 100 mg/ml was bioassayed as described in bioassay section. Methanolic extracts were also obtained as described by Bahraminejad et al. (2006). Briefly, 5 g ground sample was extracted with 100 ml methanol for 24 h by shaking on an orbital shaker at 300 rpm. Then 30 ml distilled water was added to 70 ml of the methanolic extract and lipids were removed with 100 ml n-hexane mixed at 250 rpm for 2 h. Methanolic phase was concentrated using a rotary evaporator. Finally, the residues were dissolved in 45% methanol in distilled water and a sample of extract at a concentration of 100 mg/ml for bioassay was provided.

Bioassay

Fungal bioassay was performed as previously described (Bahraminejad et al., 2008) using the paper disc method to reveal any inhibitory effect of plant crude extracts. Each autoclaved filter paper disc (6 mm diameter) was loaded with 5 × 10 µL of the crude extract (equal to 5 mg/disc). The discs were dried between each application. Negative control discs were prepared with 5 × 10 µL of the appropriate solvent, sterile water or 45% methanol. Positive control discs at concentration of 1 mg/disc were prepared with mancozeb, carboxin thiram and benomyl against *C. sativus, R. solani* and *F. oxysporum*, respectively. Loaded paper discs were placed on the growth medium about 10 mm from the margin of the growing mycelia. Five millimeter in diameter plug of each fungus was transferred to potato dextrose agar (PDA) media and incubated at 25°C in the dark until mycelia reach to approximately 25 mm from the edge of the plate. After addition of the paper discs the plates were further incubated at 25°C and radius zone of inhibition (distance between the centre of the paper disc and margin of inhibited mycelia from three different directions) was recorded. Each plate was examined for any inhibitory effect every 2 h for fast-growing fungus, *R. solani* and every 6 h for the other fungi. Four replicates plates were prepared for all extracts and controls and the experiment repeated twice.

RESULTS

Antifungal activities of the extracts are presented in Table 1. Of the 63 species tested, 21 (33%) showed activity against at least one tested fungi. 16 of 63 plant species screened (25%) measurably inhibited the mycelial growth of *R. solani*, with the most active extracts being from the following (in order for decreasing effect): *Centaurea behen, Xanthium strumarium* (Figure 1a), *Vaccaria pyramidata, Glycyrrhiza glabra, Oliveria decumbens, Sisymbrium* sp., *Avena sativa* and *Ferulago angulata,* Extracts of 10 plants (16%) inhibited the growth of *F. oxysporum*, with the extracts of *Lavandula* sp., *G. glabra, Anagallis arvensis* (Figure 1b) and *A. sativa* giving the most marked activity. 16 of the extracts (25%) exhibited activity against *C. sativus* with the greatest response from extracts of *Tribulus terrestris, Sorghum halepense, X. strumarium, G. Glabra* (Figure 1c), *V. pyramidata*, *A. arvensis* (Figure 1d) and *C. behen*. For two of the fungi, *R. solani* and *C. sativus,* some plant extracts at concentration of 5 mg/disc gave similar inhibition to the fungicides applied at concentration of 1 mg/disc. Extracts of *C. behen, X. strumarium*, *V. pyramidata, G. glabra, O. decumbens, Sisymbrium* sp., *A. sativa* and *F. angulata* showed similar or even more inhibitory effect than carboxin thiram when tested against *R. solani*. Extracts of *T. terrestris, S. halepense, X. strumarium* and *G. Glabra,* inhibited the growth of *C. sativus* similar to mancozeb.

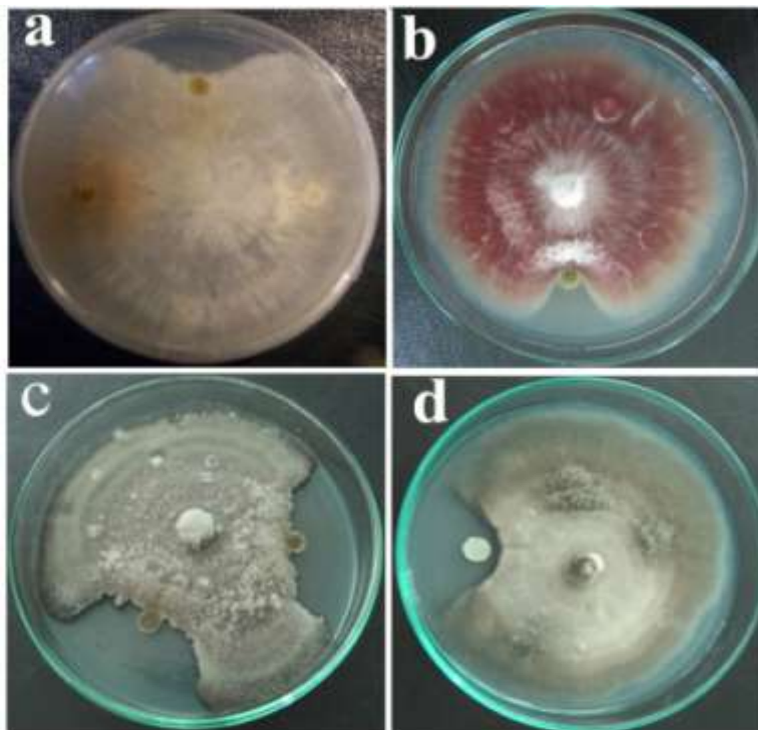

Figure 1. Inhibitory effect of plant extracts (5 mg/paper disc) of different plant pathogenic fungi on potato dextrose agar: a) *Xanthium strumarium* against *Rhizoctonia solani*, b) *Anagallis arvensis* against *Fusarium oxysporum,* c) *Anagallis arvensis* against *Cochliobolus sativus,* and d) *Glycyrrhiza glabra* against *Cochliobolus sativus.*

None of the extracts showed inhibition similar to benomyl when tested against *F. oxysporum* (Tables 1 and 2).

Data presented in Table 1 showed that the extracts of *G. glabra, R. officinalis, A. sativa, V. pyramidata, C. behen, A. arvensis* and *T. terrestris* exhibited broad-spectrum antifungal activity, inhibiting all three fungi. In this research, two different solvents were used to elicit the antifungal compounds in plant species. The results of radius inhibition zone (Table 1) which may correlate to quality and quantity of antifungal compounds indicated that antifungal compounds in the most of the plant species with anti-*Rhizoctonia*, anti-*Fusarium*, and anti-*Cochliobolus* activity were extracted by methanol.

DISCUSSION

Results indicate the presence of antifungal compounds in the different extracts (Table 1), which was in agreement with the results reported by other researchers on different pathogens (Qasem and Abu-blan, 1996; Wuben et al., 1996; Bahraminejad et al., 2008; Kostova and Dinchev, 2005). The broad spectra of inhibitory effect of *G. glabra, R. officinalis, A. sativa, V. pyramidata, C. behen, A. arvensis* and *T. terrestris* indicated that these extracts are potent antifungal plants with possible potential for the control of different fungal diseases in plants. Therefore, more research on the activity of them against the other plant pathogenic fungi would be of value. The broad antimicrobial activity of the plant species was shown to be related to the presence of saponins, alkaloids and tannins (Ndukwe et al., 2005). The antifungal activity of *A. sativa* and *T. terrestris* probably may be due to presence of saponins in their content (Crombie and Crombie, 1986; Wuben et al., 1996; Bahraminejad et al., 2008; Kostova and Dinchev, 2005). The strong activity of *R. officinalis* as a broad active plant in this study was also documented by Aye and Matsumoto (2011).

Furthermore, screening indicated that few numbers (16%) of studied plant species showed anti-*Fusarium* activity when compared to the other two fungi and all of the active extracts revealed less inhibitory effect than benomyl, thus indicating that this fungus shows more resistance to plant extracts. This kind of resistance in the fungus was previously discussed by Agrios (2005) who stated that *Fusarium* is a soil-inhabitant fungus and can adopt itself to lower level of toxic material. This may be the reason why we could not find plant extract with activity higher than benomyl. It has also been found that the methanolic extract of *L. officinalis* is the most active plant extract against *F. oxysporum.* This result was therefore in agreement with previous study showing the

Table 1. *In vitro* screening for antifungal activity (mean ± standard error, n = 4) of plant extracts at 5 mg/paper disc. Each mean was calculated from four replicates.

Plant	Family	Part used	Solvent	Plant Pathogen		
				Rhizoctonia solani	*Fusarium oxysporum*	*Cochliobolus sativus*
Ixiolirion tataricum Hall (Pall.)	Amaryllidaceae	Total	W	NI	NI	NI
			M	9.53 ± 0.3*	NI	7.00 ± 0.5
Artedia squamata L.	Apiaceae	Total	W	NI	NI	NI
			M	NI	NI	NI
Bupleurum kurdicum Boiss.	Apiaceae	Total	W	7.96 ± 0.3	WI	WI
			M	NI	WI	7.00 ± 0.1
Ferulago angulata Boiss.	Apiaceae	Flower	W	NI	NI	NI
			M	10.90 ± 0.6	NI	7.67 ± 0.9
Johrenia aromatica Rech.f.	Apiaceae	Shoot	W	NI	NI	NI
			M	NI	NI	NI
Oliveria decumbens Vent.	Apiaceae	Total	W	NI	NI	NI
			M	11.37 ± 0.6	NI	NI
Prangos ferulacea Lindl.	Apiaceae	Shoot	W	NI	NI	NI
			M	NI	NI	NI
Torilis sp.	Apiaceae	Total	W	NI	WI	WI
			M	WI	NI	NI
Carduus arabicus Jacq.	Asteraceae	Shoot	W	NI	NI	WI
			M	NI	NI	NI
Centaurea behen L.	Asteraceae	Total	W	9.67 ± 0.4	6.25 ± 0.8	7.75 ± 0.3
			M	15.25 ± 0.3	6.67 ± 0.6	9.29 ± 0.5
Crupina crupinastrum Vis.	Asteraceae	Total	W	WI	NI	WI
			M	WI	NI	NI
Cynara scolymus L.	Asteraceae	Fruit	W	NI	NI	WI
			M	WI	NI	7.29 ± 0.5
Echinops ritrodes Bunge	Asteraceae	Shoot	W	NI	NI	NI
			M	WI	NI	NI
Gundelia tournefortii L.	Asteraceae	Total	W	NI	NI	NI
			M	WI	NI	NI
Silybum marianum (L.) Gaertn.	Asteraceae	Leaf+ root	W	NI	NI	ND
			M	NI	NI	ND
Taraxacum sp.	Asteraceae	Shoot	W	WI	NI	WI
			M	WI	NI	WI
Xanthium strumarium L.	Asteraceae	Shoot	W	WI	WI	WI
			M	12.58 ± 0.9	WI	13.46 ± 0.4
Alyssum strigosum Soland	Brassicaceae	Total	W	NI	NI	NI
			M	WI	NI	NI
Conringia orientalis L.	Brassicaceae	Total	W	NI	NI	NI
			M	WI	NI	NI

Table 1 cont.

Goldbachia laevigata DC.	Brassicaceae	Total	W M	NI NI	NI NI	NI NI
Isatis lusitanica L.	Brassicaceae	Total	W M	NI NI	NI NI	NI NI
Matthiola arabica Boiss.	Brassicaceae	Total	W M	NI NI	NI WI	6.00 ± 0.4 NI
Nasturtium officinale W.T.Aiton	Brassicaceae	Total	W M	NI WI	NI NI	NI WI
Neslia apiculata Fisch., C.A.Mey. & Avé-Lall.	Brassicaceae	Total	W M	NI NI	WI NI	NI NI
Sameraria stylophora Boiss.	Brassicaceae	Total	W M	NI WI	NI NI	NI NI
Sisymbrium sp.	Brassicaceae	Total	W M	NI 11.33 ± 0.7	NI NI	NI WI
Gypsophylla sp.	Caryophyllaceae	Shoot	W M	NI NI	NI NI	NI NI
Vaccaria pyramidata Medik.	Caryophyllaceae	Total	W M	10.29 ± 0.3 12.21 ± 0.9	6.25 ± 0.3 WI	10.75 ± 0.5 10.92 ± 0.3
Capparis spinosa L.	Capparidaceae	Shoot	W M	NI NI	NI NI	NI NI
Chrozophora tinctoria A. Juss.	Euphorbiaceae	Shoot	W M	NI NI	NI AMI	NI NI
Pisum sativum L.	Fabaceae	Total	W M	NI NI	NI NI	NI NI
Glycyrrhiza glabra L.	Fabaceae	Shoot	W M	11.88 ± 0.1 11.75 ± 0.1	7.83 ± 0.4 10.83 ± 1	WI 12.54 ± 0.1
Scorpiurus muricatus L.	Fabaceae	Total	W M	NI NI	NI NI	NI NI
Muscari neglectum Guss. ex Ten	Hyacinthaceae	Total	W M	NI 5.00 ± 0.3	NI NI	NI NI
Lallemantia sp.	Lamiaceae	Total	W M	NI NI	NI NI	NI WI
Lamium amplexicaule L.	Lamiaceae	Total	W M	NI NI	NI NI	NI NI
Lavandula officinalis Caix	Lamiaceae	Shoot	W M	NI 6.34 ± 0.3	NI 10.87 ± 0.5	NI NI
Rosmarinus officinalis L.	Lamiaceae	Shoot	W M	NI 6.58 ± 0.2	NI 9.00 ± 0.2	NI 6.67 ± 0.5
Salvia sclarea L.	Lamiaceae	Shoot	W M	NI NI	NI 6.54 ± 0.6	NI NI

Table 1 cont.

Thymus kotschyanus Boiss. & Hohen.	Lamiaceae	Shoot	W	NI	NI	NI
			M	NI	WI	NI
Stachys lavandulifolia Vahl	Lamiaceae	Shoot	W	NI	NI	NI
			M	NI	NI	NI
Salvia multicalus Vahl.	Lamiaceae	Shoot	W	NI	NI	NI
			M	NI	NI	NI
Stachys inflate Benth	Lamiaceae	Shoot	W	WI	NI	NI
			M	WI	NI	NI
Gagea sp.	Liliaceae	Total	W	NI	NI	NI
			M	NI	NI	NI
Abutilon theophrasti Medik.	Malvaceae	Total	W	NI	NI	NI
			M	WI	NI	NI
Hibiscus trionum L.	Malvaceae	Shoot	W	NI	NI	NI
			M	NI	NI	NI
Olea europaea L.	Oleaceae	Fruit	W	NI	NI	NI
			M	ND	ND	ND
		Leaf + Stem	W	NI	NI	NI
			M	NI	NI	WI
Orobanche alba Rchb.	Orobanchaceae	Total	W	NI	NI	NI
			M	NI	NI	NI
Avena sativa L.	Poaceae	Shoot	W	11.13 ± 0.4	9.05 ± 0.5	NI
			M	10.54 ± 0.3	NI	NI
		Root	W	NI	NI	NI
			M	6.63 ± 0.4	8.21 ± 0.4	6.09 ± 0.3
Echinochloa crus-galli L.	Poaceae	Total	W	NI	NI	NI
			M	NI	NI	NI
Sorghum halepense (L.) Pers	Poaceae	Shoot	W	NI	NI	NI
			M	NI	NI	6.42 ± 0.7
		Rhizome	W	NI	NI	NI
			M	NI	NI	NI
		Root	W	NI	NI	NI
			M	NI	NI	13.96 ± 1
Phalaris sp.	Poaceae	Total	W	WI	WI	NI
			M	NI	NI	WI
Portulaca oleraceae L.	Portulacaceae	Shoot	W	NI	NI	NI
			M	WI	WI	NI
Anagallis arvensis L.	Primulaceae	Total	W	WI	WI	WI
			M	9.03 ± 0.3	9.3 ± 0.5	9.61 ± 0.9
Callipeltis cucullaria (L.) DC.	Rubiaceae	Total	W	NI	NI	NI
			M	NI	WI	WI
Haplophyllum perforatum (MB.) Kar. and Kir.	Rutaceae	Total	W	NI	NI	7.00 ± 0.4
			M	9.23 ± 0.5	WI	11.29 ± 0.6

Table 1 cont.

Scrophularia striata Boiss.	Scrophulariaceae	Shoot	W	WI	NI	NI
			M	NI	5.75 ± 0.4	9.58 ± 0.3
Linaria chalepensis (L) . Mill.	Scrophulariaceae	Total	W	NI	NI	NI
			M	NI	NI	NI
Scrophularia striata Boiss.	Scrophulariaceae	Total	W	NI	NI	NI
			M	NI	NI	NI
Veronica anagallis-aquatica L.	Scrophulariaceae	Total	W	NI	NI	NI
			M	WI	NI	NI
Datura stramonium L.	Solanaceae	Shoot	W	NI	NI	NI
			M	NI	NI	NI
		Root	W	NI	NI	NI
			M	NI	NI	NI
Hyoscyamus reticalatus L.	Solanaceae	Total	W	NI	NI	WI
			M	WI	NI	WI
Valerianella sp.	Valerianaceae	Total	W	WI	NI	NI
			M	NI	NI	NI
Tribulus terrestris L.	Zygophyllaceae	Shoot	W	8.13 ± 0.4	6.65 ± 0.4	8.67 ± 0.2
			M	9.04 ± 0.1	NI	4.75 ± 0.3
		Root	W	8.92 ± 0.7	NI	12.17 ± 0.1
			M	6.24 ± 0.3	NI	17.63 ± 0.1

*Mean of radius inhibition zone (mm) ± standard error; W, water; M, methanol; NI, no inhibition; WI, weak inhibition; AMI, Aerial mycelium inhibited; ND, not done.

Table 2. Inhibitory effect of fungicides used as positive controls (mean ± standard error, n = 6) at 1 mg/ disc.

Fungicide	**Plant Pathogen**		
	Rhizoctonia solani	***Fusarium oxysporum***	***Cochliobolus sativus***
Mancozeb	-	-	12.22 ± 0.8
Carboxin thiram	10.44 ± 0.4	-	-
Benomyl	-	25.22 ± 0.5	-

antifungal effect of *L. officinalis* against mycelial growth of *F. oxysporum* (Pawar and Thaker, 2007). In this study, *X. strumarium* with common name cocklebur (Asteraceae) also showed significant anti-*R. solani* and anti-*C. sativus* properties. The ant-*Phytophthora drechsleri* properties of cocklebur have been previously reported (Kim et al., 2002; Koko, 2007; Yanar et al., 2011). Kim et al. (2002) extracted and purified an anti-*P. drechsleri* compound from *X. strumarium.* This compound was identified as a sesquiterpene lactone called deacetyl xanthumin. The results of the present work confirmed the presence of toxic substances of *X. strumarium* shown in previous

study.

In this study, three of the seven plant species in Apiaceae, two of the eight plant species in Asteraceae, two of the nine plant species in Brassicaceae, three of the nine tested plant species in Lamiceae and three of four plant species in Poaceae showed antifungal activity. Although, the number of tested plant species in each family was not in high frequency, it can be concluded that inhibitory effect is not family dependent. This finding is in agreement with the results reported by Qasem and Abu-Blan (1996) on some other pathogens. However, the taxonomic distribution for phytoalexin production was well reviewed by Grayer (1994) and is not in agreement with the results of our study. He reported a high frequency of plant species of Fabaceae and low frequency of Rosaceae with antimicrobial activity. Differences in the toxicity of the extracts could be due to their solubility in water and methanol and results might be influenced by the solubility of the active substances in the solvent with higher solubility of the most active plant extracts in the water or methanol. Therefore, it can be concluded that different plants need different extracting solvent. The finding of this study supported the observation of Eloff (1998) who ranked extractants based on their ability to solubilize antimicrobial compounds from plants, biohazards and ease of removal of solvents from fractions. Eloff ranked methanol in second to methylene dichloride and superior to ethanol and water. However, application of the other solvents could be useful to extract more toxic metabolites from the plants reported in this work.

As Chitwood (2002) stated, the results of these kind of research could help to develop new natural fungicide, chemically synthesized derivatives or to grow the plants with antifungal activity in a crop rotation program. These results will also help to find out the active metabolites in active plants and subsequently used in reverse genetic engineering from metabolites to genes. Regarding the allelopathic properties of oats (*Avena sativa*), oat can be grown in a crop rotation program to suppress and break the cycle of soil-borne plant pathogenic fungi (Schrickel, 1986). Therefore, oat as a known crop plant in Iran could help to reduce the severity of soil borne diseases in wheat farms. These results and the acceptable percentage of the plants with antifungal activity (33% in this research) indicated that the flora in the west of Iran can be regarded as a rich source of plants with antifungal activity. These findings encouraged us to continue screening more plant species for antifungal agents.

The results of this study may form the basis of further investigation on fractionation for finding active fractions, the effect of origin of growth on the quality and quantity of active compounds, the amount of bioactive compounds in different plant parts and finally *in vivo* application of the extracts. Therefore, further investigations are being conducted on *X. strumarium*, *A. arvensis*, and *T. terrestris* as they showed more durability of inhibition and wide range of effects against different fungi.

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