Australian Journal of Crop Science AJCS
AJCS 6(2):255-260 (2012) ISSN:1835-2707

Evaluation of inhibitory effects of extracts of plants from western Iran against *Phytophthora drechsleri*

S. Bahraminejad*, S. Abbasi, S.M. Maassoumi, S. Tabein

Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran

***Corresponding author: sohbah72@hotmail.com**

Abstract

Crude aqueous and methanol extracts of 121 plant species from 41 families, collected from the west of Iran, were screened for antifungal activity against mycelial growth of *Phytophthora drechsleri.* The bioassay used was based on the paper disc diffusion method with four replicates. Extracts of 38 of 121 (about 31%) plant species had inhibitory activity against this phytopathogenic fungus, among which 23 species measurably inhibited the growth of *Phytophthora drechsleri*. A methanol extract of *Xanthium strumarium* had the strongest inhibitory activity (17.79±1.35 mm) against *P. drechsleri* followed by extracts of *Glycyrrhiza glabra, Verbascum sp., Hypericum perforatum, Centaura depressa, Centaura sp., Lamium amplexicaule, Haplophyllum perforatum.* An investigation of the efficacy of extracts of different plant parts on mycelial inhibition of *P. drechsleri*, using the paper disc method, indicated that the inflorescence and fruits of cocklebur (*Xanthium strumarium*) showed significantly more inhibitory effect than the other plant parts against the fungus. Two common species of cocklebur, *X. strumarium* and *X. spinosa,* grown around the city of Kermanshah, inhibited mycelial growth of the tested fungus, but extracts of *X. strumarium* had significantly more inhibitory effect against *P. dreschleri* than *X. spinosa.* The results of fractionation of leaf, fruit and inflorescence by thin layer chromatography (TLC) indicated that yellow and blue fractions (under UV) with relative fronts (Rf) equal to 0.93 and 0.98 of leaf, fruit and inflorescence fractions exhibited the highest inhibitory effect against *P. dreschleri.* These results suggest that cocklebur has potential for control of *P. dreschleri* and further green house and farm studies are recommended.

Keywords: Anti-*Phytophthora* activity; Iranian plants; Paper disc; Thin layer chromatography; *Xanthium strumarium.*
Abbreviation: IPM-Integrated Pest Management, TLC- Thin Layer Chromatography, UV-Ultra Violet, Rf- Relative to front, PDA-Potato Dextrose Agar, CRD- Completely Randomized Design, DMRT- Duncan's new Multiple Ranges Test.

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 for pest and disease control. In spite of hazards associated with chemical pesticide application including problems of public health, environmental pollution, toxic effects on non-target organisms and development of resistance in pest and disease agents (Rai and Carpinella, 2006; Kagale et al., 2004), it is believed that fungicides will remain an essential tool for the control of plant diseases and their use should be optimized under integrated pest management programs (Gullino et al., 2000). IPM for conserving agro-ecosystems includes the use of pest-resistant cultivars, holding pests at a tolerable level and using environmentally safe methods such as natural products (Rai and Carpinella, 2006). Given the effect of the origin of plant species and genetic diversity on chemical composition, studies screening for novel antifungal compounds in plants from different parts of the world are needed. In this research, we screened plants from the west of Iran for anti-*Phytophthora* activity. Some Iranian plants were screened for antimicrobial activity previously (Sardari et al., 1998; Fazly Bazzaz et al., 1997; Fazly Bazzaz and Haririzadah 2003; Shahidi Bonjar et al., 2004), but these investigations were focused on screening plant extracts against agents of disease in humans. There are few antifungal screening studies for the phytopathogenic fungi in Iran. *Phytophthora* is a plant pathogen with about 50 species causing wide variety of disease on large number of hosts (Chaube and Pundhir, 2005). It causes root, stem and fruit rots resulting in severe reductions in crop yield. Use of metalaxyl+mancozeb as a seed treatment has been recommended to control effectively the disease caused by *P. drechsleri*, the causative agent of pigeon pea blight, up to 15 days after sowing (Chaube and Pundhir, 2005). In addition, seed and soil treatments of isolates of *Pseudomonas fluorescens* significantly protect Cantaloupe plants from attack of *P. drechsleri* (Tabarraei et al., 2011). Preliminary *in vitro* experiments conducted with several plant crude extracts showed that some of extracts could effectively control *P. drechsleri.* Plants such as *Zataria multiflora, Pinus halepensis, Carum carvi* (Abdolmaleki et al., 2010), *Cinnamomum zelanicum* (Abdolmaleki et al., 2008) and *Xanthium strumarium* (Kim et al., 2002) significantly suppressed the mycelial growth of *P. drechesleri.* Methanol and ethyl acetate extracts of yellow oleander (*Thevetia peruviana*) had a range of inhibitory effects on the mycellial growth of different strains of *P. megakarya*, the agent of black pod disease (Ambang et al., 2011). *In vivo* experiments using crude plant extract against *Phytophthora* blight of pepper caused by *P. capsici* showed

that the severity of disease was reduced by cabbage, garlic and alfalfa extracts (Demirci and Dolar, 2006). The objectives of the present study, as part of larger screening program, were to identify plant sources with anti-*Phytophthora* activity, determine which plant parts have higher activity and to find the active fractions.

Results

Plant screening for inhibitory activity against P. drechsleri

Crude methanol and aqueous extracts of 121 Iranian plant species from 41 families were tested against *Phytophthora drecesleri* at a concentration of 100 mg/ml and 5 mg/paper disc. Anti-*Phytophthora* activity of the extracts in terms of radius inhibition zone is reported in Supplementary Table. Of the 121 plant species tested, 38 (about 31%) showed activity against the fungus. Among these, 23 species measurably inhibited the mycelial growth of *P. drechsleri.* Results indicated that methanol extract of *Xanthium strumarium* showed the maximum activity (17.79±1.35 mm) against *P.drechsleri* (Fig 1.) followed by *Glycyrrhiza glabra, Verbascum* sp., *Hypericum perforatum, Centaura depressa, Centaura* sp., *Lamium amplexicaule, Haplophyllum perforatum, C.behen, Rosmarinus officinalis, Portulaca oleraceae , Sisymbrium* sp., *Datura stramonium, Papaver dubium, Consolida* sp., *Fritillaria imperialis, Anchusa italica, Taraxacum* sp., *Lavandula* sp., *Tribulus terrestris, Olea europaea, Vaccaria pyramidata* and *Avena sativa.*

The inhibitory activity of different parts of X.strumarium against Phytophthora drechsleri

To find out which part had the highest anti-*Phytophthora drechsleri* activity, root, stem, leaves, inflorescence and fruits of *X. strumarium* were separated from each other and extracted by methanol after drying. The results of the paper disc method indicated that extracts of inflorescence and fruit remarkably inhibited the mycelial growth of *P. drechsleri.* The inflorescence had significantly more inhibitory effect than leaves, and roots of *X. strumarium* did not inhibit the growth of *P. dreschleri* (Table 1.)

Anti-Phytophthora drechsleri activity of X. strumarium at different stages of growing

Fluctuations in antifungal contents of *X. strumarium* during different plant growth stages were investigated. The inhibitory effect of crude extracts obtained from different stages against *P. drechsleri* was used as an indicator of antifungal contents. The results indicated that the amount of inhibition was significantly lowest at the seedling stage when the cotyledonous leaves appeared and with growth of the plant only subtle fluctuation was seen (Fig 2).

Anti-Phytophthora drechsleri activity of X. strumarium and X. spinosa

The anti-*Phytophthora drechsleri* activity of crude extracts of *X. strumarium* and *X. spinosa* was significantly different, with the extracts obtained from *X. strumarium* having significantly more inhibitory activity than extracts obtained from *X. spinosa* (data not shown).

Fractionation of the crude extract

The crude leaf, fruit and inflorescence methanol extracts of *X. strumarium* were fractionated to ten, nine and nine fractions, respectively, by TLC under UV light (254/365 nm). After recovery, these fractions were exposed to grown mycelial of *P. drechesleri* in the concentration of 1 mg/paper disc. Although the two slowest moving fractions showed the inhibition, the last fraction of each plant part was the most active against this fungus; and the last fraction of the leaf extract was the most active when compared with that of the fruits and inflorescence. These fractions, from the different plant parts, were similar in colour with a narrow yellow band (Rf=0.93) and a blue band (Rf=0.98).

Discussion

Initial screening of plants for possible anti-*Phytophthora drechsleri* activity began with aqueous and methanol extractions as stated by Cowan (1999); and followed by logical pathway. After the initial screening, *Xanthium strumarium* was selected as the plant showing most inhibitory activity. After determining which were the strongest plant extracts, experiments were made to find the most active part of the plant, growth stage/s, plant species and plant fraction/s. Several studies showed the potential of crude extracts as antifungal agents (Lee et al., 2007; Erturk, 2006; Sardari et al., 1998). In the present research, crude methanol and aqueous extracts of 121 Iranian plant species were tested against *Phytophthora drechsleri*. Due to the importance of screening plant crude extracts as the first step and the importance of bioactive crude extracts as ecofriendly agents, plants collected from the west of Iran were screened against this destructive fungus. At present, metalaxyl is seen as an effective systemic fungicide to control the disease caused by *Phytophthora* (Ware and Whitacre, 2004), but two important points are arose when it is applied, one is the need for timely application of the fungicide to get effective control (Kim et al., 2002) and the other is creation of new strains of pathogen tolerant to metalaxyl (Kim et al., 2002). Therefore, further research is needed to establish new, safe, alternative methods for fungal control. All *Centaurea* species tested were shown to be active against *P. drechsleri*. The antifungal activity of *Centaurea* species against phytopathogenic fungi other than *P. drechsleri* was reported by Skaltsa et al. (2000), Panagouleas et al. (2003) and Karamenderes et al. (2006). However, this antifungal activity depends on the tested species of *Centaurea* and the solvent used (Cansaran et al., 2010). Nine different compounds isolated from the aerial parts of C. *thessala* ssp. *drakiensis* and *C. attica* ssp. *attica* were effective against fungi (Skaltsa et al., 2000). The genus *Haplophyllum* comprises about 50 species and is distributed from Africa to Eurasia. About 30 species of this perennial plant grow in Iran and 14 of them are endemic to it. *Haplophyllum* contains several quinoline alkaloids (Staerk et al., 2009) and lignan lactones (Sheriha et al., 1987). In this study, it was shown that *H. perforatum* (syn. *H. acutifolium*) collected from Homail- a site 65 km from Kermanshah, Iran- contains strong antifungal activity. Our results are in accordance with the previous findings reported by Cantrell et al., 2005 who found that this plant possesses antifungal activity and quinoline alkaloids especially flindersine are responsible for this activity.

Table 1. The inhibitory effect (mean ± standard error) of different parts of *Xanthium strumarium* on mycelia growth of *Phytophthora drechsleri* compared using Duncan's test at p≤0.05. Each mean was calculated from five replicates.

Plant Part	Zone of Inhibition (mm)
Inflorescence	17.50 ± 0.27 [a]
Fruit	16.33 ± 0.78 [ab]
Leaf	15.93 ± 0.83 [b]
Stem	6.30 ± 0.30 [c]
Root	0

Means followed by the same letters are not significantly different at P≤0.05.

Fig 1. Inhibitory effect of crude extract obtained from *Xanthium strumarium* (5 mg/paper disc) on mycelial growth of *Phytophthora drechsleri*

The results indicated that *Sisymbrium* sp. and *Lamium sp.* had strong inhibitory effects against the fungus. *Sisymbrium* has shown to have an allelopathic effect on seed germination of neighboring plants and spore germination of mycorrhizal fungi (Bainard et al., 2009). Strong antifungal activity of *Lamium tenuiflorum* against *Candida albicans* was reported by Basaran (2009). While several plant extracts tested had a high level of inhibition at a single concentration (5mg/paper disc), the plant extract with a significant and noticeable effect on *P. drechsleri* compared to the other extracts and positive control (mancozeb) was selected in the screening experiment for further investigations. *Xanthium strumarium,* common name cocklebur (Asteraceae), an annual weed prevalent in the west of Iran was selected. It is resistant to drought and is a common weed in pastures and fields. The antimicrobial properties of cocklebur have been previously reported (Bahraminejad et al., 2011; Cerdeiras et al., 2007; Nariman et al., 2004; Lavault et al., 2005; Koko, 2006; Kim et al., 2002). Anti-*Candida albicans* activity by *X. strumarium* was reported by Shahidi Bonjar et al., (2004). Our data confirmed the findings of a previous study which reported that the extracts of *X. strumarium* effectively inhibited the mycelial growth and zoospore germination of *P. drechsleri* (Kim et al., 2002). Kim et al. (2002) purified the anti-*Phytophthora* compound in cocklebur as deacetyl xanthumin. In this study, a methanol extract of cocklebur (*X. strumarium*) completely inhibited the mycelial growth of *P. drechsleri* when the disc diffusion method was used, and caused about 83% inhibition when the agar dilution method was used. Moreover, this inhibition was durable, i.e., the mycelium had not covered the impregnated paper disc after a week. Fractionation by TLC and bioassay resulted in a yellow and a blue coloured fraction (under UV) on developed TLC plates and all of the active parts of the plant had an inhibitory effect on the tested fungus. These results indicate that the antifungal compound probably is not the only compound as reported by Kim et al., (2002) and antifungal activity in *X. strumarium* could be due to the existence of either two different fractions or the two fractions and their interactions. As any part of the plant may contain active compounds, an experiment was carried out using the disc diffusion method. It showed that roots do not contain anti-*Phytophthora* compounds. Least inhibitory activity was obtained from plants at the cotyledonous stage (young seedlings) and it fluctuated a little during growth of the cocklebur. This detailed study helps our understanding of the changes in the active compounds in terms of plant parts, species and age. This is the first report on some physiological aspects of the antifungal compounds of the *X. strumarium*.

Both species of *Xanthium* tested for inhibition of *P. drechsleri* growth inhibited mycelial growth of the fungus, suggesting that this property may exist in other species of *Xanthium;* as reported by Qi et al. (2008) for *X. sibiricum* , Eftekhar et al. (2007) for *X. brasilicum* and Cerdeiras et al. (2007) for *X. cavanillesii*. Because of the diverse range of antifungal compounds with different properties, they may not be completely extracted by a single solvent (Wojcikowski et al., 2007). In this research two different solvents were used to elicit the antifungal compounds in plant species. Measurements of the radius inhibition zone (Supplementary Table) may correlate to quality and quantity of antifungal compounds and indicated that antifungal compounds in the most plant species with anti-*Phytophthora* activity were extracted by methanol. This finding 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 second to methylene dichloride and superior to ethanol and water. Eftekhar et al. (2007) found that methanol and a solvent mixture were the best solvents to elicit antimicrobial compounds from *X. brasilicum*. These results and the encouraging percentage of plants with antifungal activity (31% in this research) indicated that the flora in the west of Iran can be regarded as rich sources of plants with antifungal activity. These findings persuaded us to continue screening more plant species.

The results of this study could form the basis for further investigation of fractionation for finding active fractions, the effect of origin of growing on the quality and quantity of active compounds, the amount of bioactive compounds in different plant parts and finally *in vivo* application of extracts will be considered.

Materials and methods

Plant material and fungi

One hundred and twenty one plant species from 41 families were collected from different parts of the provinces of Kermanshah and Hamadan located in the west of Iran (Supplementary Table). As a part of wider screening program, plants were randomly collected to increase the chance of finding plants with bioactive extracts. The plants were identified by staff at the Herbarium at Razi University, College of Agriculture and scientific names were checked in the International Plant Names Index

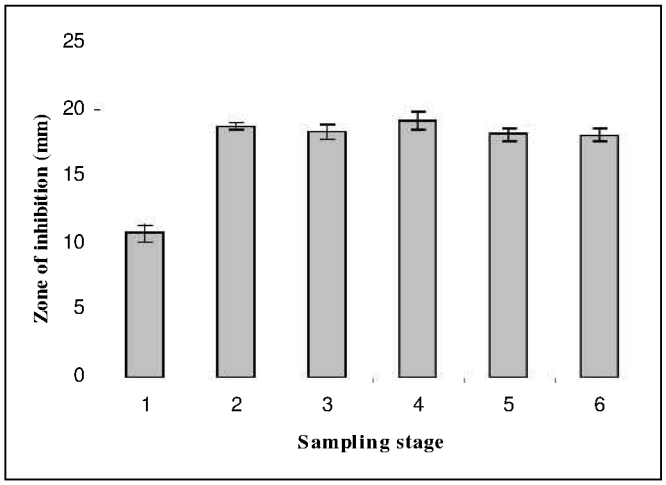

Fig 2. The mean inhibitory effect of *Xanthium strumarium* harvested at different stages of life cycle against mycelia growth of *Phytophthora drechsleri* compared using Duncan's test at $p \leq 0.05$. Each mean was calculated from four replicates. 1, 2, 3, 4, 5, 6 stages are cotyledonous leaves, five main leaves, 10 main leaves, flowering, fruiting and physiological maturity, respectively.

(http://www.ipni.org/ipni/plantnamesearchpage.do). Each sample was cleaned, shade dried and ground to a fine powder with a coffee grinder before methanol or aqueous extraction. An isolate of *Phytophthora drechsleri* was provided by the Agriculture and Natural Resources Research Centre of Kermanshah. It was isolated from sugar beet and had proven pathogenicity.

Preparation of plant crude 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 powdered materials and bringing the mixture to the boil. It was then allowed to cool to laboratory temperature. Four hours after taking the mixture off heat, it was filtered. The extract was then concentrated using a rotary evaporator. A sample of extract at concentration of 100 mg/ml was provided. The Methanol extract was obtained as described by Bahraminejad et al. (2006).

Bioassay

The fungal bioassay was performed as described by Bahraminejad et al. (2008). In this study, the paper disc method was used to test for any inhibitory effect of crude plant extracts. Positive control discs with the fungicide mancozeb at a concentration of 1 mg/disc were tested against *P. drechsleri* in order to determine the effectiveness of the crude extracts. A five millimeter diameter plug of the fungus was transferred to potato dextrose agar (PDA) media and incubated at 25°C in the dark until the fungal mat was approximately 25 mm from the edge of the plate. Then, loaded paper discs were placed on the growth medium about 10 mm from the margin of the growing colony. Inoculated plates were incubated at 25°C and radius of the zone of inhibition (distance between the centre of the paper disc and margin of inhibited colony from three different directions) was recorded in millimeters. Each plate was examined for inhibitory effects every six hours. All plant extracts and controls were tested in four replicates and the experiment was repeated twice.

The inhibitory activity of different parts of X. strumarium against Phytophthora drechsleri

Roots, stems, leaves, inflorescence and fruits of *X. strumarium* were separated after drying in the shade. The plant parts were ground and extracted by methanol as mentioned above. Grown mycelia of *P. drechsleri* were exposed to impregnated paper discs at a concentration of 5mg per paper disc. The experiment had five replicates. A completely randomized design (CRD) was used to show the differences among plant parts for antifungal activity. Duncan's New Multiple Ranges Test (DMRT) was applied to determine any significant differences when means of treatments (plant parts) were compared.

Anti-Phytophthora drechsleri activity of X. strumarium at different stages of growing

Seeds of *X.strumarium* were sown in a four-row plot with 75 cm inter row spacing and 20 cm plant to plant spacing. The first plants were harvested at the cotyledonous leaf stage. Later samples were harvested in consecutive months so that we had stages as follows: cotyledonous leaf stage, 5 leaf stage, 10 leaf stage, flowering, fruiting, and physiologically matured seeds. Above ground parts of plants at each stage were cut and dried in the shade. They were ground and stored. Five grams of each sample was used for extraction. After methanol extraction, grown mycelia of *P. drechsleri* were exposed to the extracts in four replicates. A completely randomized design (CRD) was used. DMRT was also applied to determine any the significant differences when means of treatments (plant growth stages) were compared.

Anti-Phytophthora drechsleri activity of X. strumarium and X. spinosa

Above ground parts of two common species growing in the west of Iran, *X. strumarium* and *X. spinosa* were collected (one mature plant of each species) and extracted with methanol. The grown mycelia of *P. drechsleri* were exposed to four replicates of the respective extracts to see any differences between the two species in terms of antifungal activity. An unpaired t-test was used to show any significant difference between the two species for antifungal activity.

Fractionation of the crude extract

Fractionation of the crude extract of *X. strumarium* was carried out using preparative thin layer chromatography (TLC) using aluminium-backed plates (0.20 mm, silica 60, Merck KGA) developed with ethyl acetate : methanol : water (31 : 4 : 4 v / v / v) as the solvent. The developed chromatograms were examined under UV light (254/365 nm). Observed fractions having similar Rf, relative to the front were pooled, and materials were recovered from the silica gel. Eluted compound or compounds at a concentration of 1 mg/paper disc was tested for ant-*Phytophthora drechsleri* activity using the paper disc diffusion method. This experiment was performed with three replicates and values are expressed as mean ± standard deviation.

Acknowledgements

The authors would like to thank Dr Mahyar Shaikholeslami for providing fungal isolates. Special thanks to Dr Kerrie Davies from University of Adelaide for her valuable editorial comments and helpful revision to improve the manuscript. This research was financially supported by Razi University of Kermanshah.

References

Abdolmaleki M, Bahraminejad S, Abassi S, Mahmodi SB (2010) Inhibitory effect of some plant extracts on mycelial growth of *Rhizoctonia solani* and *Phytophthora drechsleri*, sugar beet root rot agent. Journal of Sugar Beet 25: 193-205

Abdolmaleki M, Salari M, Bahraminejad S, Panjekeh N, Abassi S (2008) Antifungal activity of cinnamon (*Cinnamomum zelanicum*) crude extracts against some phytopathogenic fungi. Iranian Journal of Plant Pathlogy 44: 255-261

Agrios GM (2005). Plant Pathology. 5th ed. Academic Press, New York, NY.

Ambang Z, Ngoh Dooh JP, Essono G, Bekolo N, Chewachong G, Asseng CC (2010) Effect of *Thevetia peruviana* seeds extract on *in vitro* growth of four strains of *Phytophthora megakarya.* Plant Omics Journal 3:70-76.

Bahraminejad S, Abbasi S, Fazlali M (2011) In vitro antifungal activity of 63 Iranian plant species against three different plant pathogenic fungi. Afr J Biotechnol 10 (72): 16193-16201

Bahraminejad S, Asenntorfer RE, Riley IT, Schultz CJ (2008) Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots oats (*Avena sativa* L.). J Phytopathol 156:1-7.

Bahraminejad S, Asenntorfer RE, Riley IT, Zwer P, Schultz CJ, Schmidt O (2006) Genetic variation of flavonoid defense compound concentration in oat (*Avena sativa* L.) entries and testing of their biological activity. Paper presented at 13th Australasian plant breeding conference, Christchurch , New Zealand 18-21 May.

Bainard LD, Brown PD, Upadhyaya MK (2009) Inhibitory effect of tall hedge mustard (*Sisymbrium loeselii*) allelochemicals on rangeland plants and arbuscular mycorrhizal fungi. Weed Sci.57:386-393

Basaran D (2009) Antifungal activity of *Lamium tenuiflorum* against some medical yeast *Candida* and *Cryptococcus* species. Pharm Biol 47: 467-470.

Cansaran A, Do NM, Öztekin M, Acar G (2010) Antimicrobial activity of various extracts of *Centaurea cankiriense* A. Duran and H. Duman. Afr J Microbiol Res 4: 608-612.

Cantrell CL, Schrader KK, Mamonov LK, Sitpaeva GT, Kustova TS, Dunbar C, Wedge DE (2005) Isolation and identification of antifungal and antialgal alkaloids from *Haplophyllum sieversii*. J Agr Food Chem 53:7741-8.

Cerdeiras MP, Albores S, Etcheverry S, Lucian V, Soubes M, Vazquez A (2007) Antimicrobial activity of *Xanthium cavanillesii* extract. Pharm Biol 45: 251-254.

Chaube HS, Pundhir VS (2005) Crop diseases and their management. Prentice-Hall of India. PP 702

Cowan, MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12:564-582

Demirci F, Dolar FS (2006) Effects of some plant materials on *Phytophthora* blight (*Phytophthora capsici* Leon) of pepper. Turk J of Agric For 30: 247-252

Eftekhar F, Nariman F, Habibi Z, Mohammadi N (2007) Antibacterial activity of the aerial extracts from *Xanthium brasilicum*. Iranian J Pharmaceut Res 6:65-68

Eloff JN (1998) Which extractant should be used for the screening and isolation of ntimicrobial components from plants? J Ethnopharmacol 60:1–8

Erturk O (2006) Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Biologia, Bratislava 61: 275-278

Fazly Bazzaz BS, Haririzadeh G, Imami SA, Rashed MH (1997) Survey of Iranian Plants for Alkaloids, Flavonoids, Saponins, and Tannins [Khorasan Province]. Int J Pharmacogn 35 :17-30

Fazly Bazzaz BS, Haririzadeh G (2003) Screening of Iranian plants for antimicrobial activity. Pharm Biol 41 : 573-583

Gullino ML, Leroux P, Smith CM (2000) Uses and Challenges of novel compounds for plant disease control. Crop Prot19:1-11

Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R (2004) Antimicrobial activity and Induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *Oryzae*. Physiol Mol Plant Pathol 65: 91-100

Karamenderes C, Khan S, Tekwani B, Jacob MR, Khan IA (2006) Antiprotozoal and antimicrobial activities of Centaurea species growing in Turkey. Pharm Biol 44: 534–539

Kim DK, Shim CK, Bae DW, Kawk YS, Yang MS, Kim HK (2002) Identification and biological characteristics of an antifungal compound extracted from cocklebur (*Xanthium strumarium*) against *Phythopthora drechsleri*. Plant Pathology J 18: 288-292.

Koko WS (2006). Antimalarial activity of *Xanthium brasilicum* Vell in vitro, in vivo and toxicological approach. Recent Progress in Medicinal Plants (Natural Products Issue) 15: 1-10.

Lavault M, Landreau A, Larcher G, Bouchara JP, Pagniez F, LePape P, Richomme P (2005) Antileishmanial and antifungal activities of xanthanolides isolated from *Xanthium macrocarpum.* Fitoterapia. 76 : 363-366.

Lee SH, Chang KS, Su MS, Huang YS, Jang HD (2007) Effects of some Chinese medicinal plant extracts on five different fungi. Food Control 18: 1547-1554

Nariman F, Eftekhar F, Habibi Z, Falsafi T (2004). Anti-*Helicobacter pylori* activities of six Iranian plants. Helicobacter 9 : 146-151

Oerke EC, Dehne HW, Schonbeck F, Weber A (1994) Crop protection and crop production. Elsevier, Amsterdam, 808pp.

Panagouleas C, Skaltsa H, Lazari D, Skalsounis AL, Sokovic M (2003) Antifungal activity of secondary metabolites of *Centaurea raphaniana* ssp. *mixta* growing wild in Greece. Pharm Biol 41: 266-270

Qi L, Liu L,Yu P, Chen F (2008) Preliminary studies on antifungal activity of *Xanthium sibiricum* and the endophytic fungi. Agricultural Science and Technology 9 : 144-148.

Rai MK and Carpinella C (2006) Naturally occurring bioactive compounds: a new and safe alternative for control of pests and microbial diseases, Elsevier Publication, Netherland

Sardari S, Amin G, Micetich GR, Daneshtalab M (1998) Phytopharmaceuticals. Part 1. Antifungal activity selected Iranian and Canadian plants. Pharm Biol 36 : 180-188.

Shahidi Bonjar GH, Aghighi S, Karimi Nik A (2004) Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J Biol Sci 4: 405-412

Sheriha GM, Abouamer K, Elshtaiwa BZ, Ashour AS, Abed FA, Alhallaq HH (1987) Quinoline alkaloids and cytotoxic from lignans from *Haplophyllum tuberculatum*. Phytochemistry 26: 3339-3341

Skaltsa H, Lazari D, Panagouleas C, Georgiadou E, Garcia B, Sokovic M (2000) Sesquiterpene lactones from *Centaurea thessala* and *Centaurea attica*. Antifungal activity. Phytochemistry 55: 903-908

Staerk D, Kesting JR, Sairafianpour M, Witt M, Asili J, Emami SA, Jaroszewski JW (2009) Accelerated dereplication of crude extracts using HPLC–PDA–MS–SPE–NMR: Quinolinone alkaloids of *Haplophyllum acutifolium*. Phytochemistry 70: 1055–1061

Tabarraei M, Amini J, Harighi B (2011) Effects of *fluorescent Pseudomonas* for control of damping-off disease of cantaloupe caused by *Phytophthora drechsleri*. Aust J Crop Sci 51427-1433.

Ware G, Whitacre D (2004) The Pesticide Book . 6 ed. USA, MeisterPro, Willoughby,OH. 488 p

Wojcikowski K, Stevenson L, Leach D, Wohlmuth H, Gobe G (2007) Antioxidant capacity of 55 medicinal herbs traditionally used to treat the urinary system: A comparison using a sequential three-solvent extraction process. J Altern Complem Med 13: 103-109.