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Original Article

Antifungal Effects of Two Medicinal Plant Native to Iran

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ABSTRACT

Objective: In order to use natural compounds in controlling plant pests and diseases, many researchers in recent years have studied the antifungal effects of essential oils and plant extracts. The purpose of this study was to investigate the chemical composition and antifungal effects of *Echium khuzestanium* and *marubbium anisodan* extracts. **Methods:** After extraction and preparation of different concentrarion from extracts, antifungal effect on 4 plant fungi was stadied by disc diffusion method. chemical composition of plants was checked by GC/MS. **Results:** According to the results, 86 kinds of chemical compounds found in *M.anisodan* extract. Furfural, steroids, vitamin B and flavonoids are the main compounds of *M.anisodan*. 46 kinds of chemical compounds found in methanol extract of *E.khuzestanium*. there are mucilage, fatty acids, flavonoid and diterpenes in flower of *E.khuzestanium*.

1.INTRODUCTION

In order to use natural compounds in controlling plant pests and diseases, many researchers in recent years have studied the antifungal effects of essential oils and plant extracts. Frequent and indiscriminate use of chemical pesticides, in addition to the environmental pollution was resulted in the phenomenon of resistance to pesticides (naranayami etal, 2002). Essential oils and plant extracts contain compounds with different biological activities such as Antimicrobial effect (Rodriguez etal, 2005). In this regard, many researchers studied the antibacterial, antifungal and insecticidal effects of essential oils and plant extracts (Alam et al., 2007 Muyima et al., 2004; Pitaroki et al., 2003; Shimoni et al, 1993). Secondery metabolites had important ecological roles in plant defense responses. Many metabolites are involved in plant defense against pests and diseases (Cowan, 1999). Identify and evaluate these metabolites can effectively help to control pests and diseases in plants. The amount and type of metabolites in plant depends on environmental and geographic conditions of habitat (azlan etal, 2003). *Echium khuzestanium* is a biennial plant of Boraginaceae which grow in southwest of Iran (Khatamsaz, 2002). Boraginaceae is the most known source of gamma-linoleic acid that have high nutritional and medicinal value (Horrobin, 1992). There are naphthoquinonone pigments such as alkanin and shikonin derivatives in roots of at least 150 species of Boraginaceae. Alkanin(S enantiomer) and shikonin(R enantiomer) and their derivatives have a lot of medicinal properties like antibacterial, anticancer, antioxidant, anti-inflammatory properties and wound healing (Papageorgiou et al, 2006a, 2006b, 2002, 1999). *Marrubium anisodan* is a plant of Lamiaceae. There are various activity such as

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antioxidant (Weel et al, 1999) and anti-inflammatory (Shahpaz et al, 2002). The purpose of this study was to investigate the chemical composition and the antifungal effect of Echium khuzestanium and marrubium anisodan extract.

2. MATERIALS AND METHODS

2.1.Plant material

Arial part of *Marrubium anisodan* was collected from medicinal plant garden of Hamadan. *Echium khuzistanium* cultivated in greenhouse. Seed of this plant was brought from Alhaii region around Ahwaz (southwest of Iran). After collection, the plants were identified by botanist.

2.2.Extraction of plant material

The samples were dried at room temperature and dark condition and further ground in a mortar. About 10 grams of each plant powder extracted in 100 ml of methanol by maceration (48h). The solvent was concentrated at temperature below 40°C and the resulting extracts were used for determination of antifungal effect and chemical composition(Ebrahimzade etal, 2008).

2.3.Evaluation of antifungal effect

Fusarium oxysporum ،*Fusarium solani* ،*Pythium aphanidermatum* و *Rhizoctonia solani* were used to examine antifungal effect. Fungi were obtained from the Department of Pathology, Faculty of Agriculture, University of Bu-Ali Sina. To evaluate the inhibitory effects was used doses of 100, 200, 400 and 600 mg extract per ml of a methanol, ethanol and hydro alcoholic solvent. The antifungal activity was tested by disc diffusion method . The potato dextrose agar plates were inoculated with each fungal culture (7days old). Tablets with 6mm diameter was prepared by Cork borer from growing colony margin a week and was placed in the middle of PDA medium. The filter paper discs (5 mm diameter) impregnated with different concentrations of the extracts were placed on test organism-seeded plates. Rhizoctonia colony diameter after 32 hours and three other fungi after 72 h was about three cm when incubated at 25˚c. After this step, the extract impregnated discs were placed at a certain distance from the edge of the fungus grown(1cm for Rhizoctonia and 1.5 cm for three other fungi). Then, at different intervals, the radius of the inhibition zone was recorded and mean values were considered in the calculations. Three replications were tested. In a completely randomized design, factorial experiments were performed. Expriments were performed in in the Laboratory of Biotechnology, Faculty of Agriculture, University of Bu-Ali Sina.

2.4. Gas chromatography-mass analyzer

Chemical composition of extracts was identified by GC/MS (Agilent 6890N gas chromatography coiled with Agilent 5973N mass detector). 1µl of each extract was injected. The separation of extract was performed using a HP-5 column of 30m in length and 0.25 mm in diameter and 0.25 µm in stationary phase thickness. The analysis conditions were:

Temperature program of analysis

Rate(˚C/min)	Temperature	Hold (min)
-	60.00	0.00
5.00	150.00	0.00
10.00	250.00	0.00

Solvent delay was 5 min and the identification of compound was based on comparing their mass spectra with those recorded in the Wiley 7n mass spectra database and with literature reports.

3. RESULT

To analyze results accurately and given that chemical composition of these plants are not detected, after extraction, chemical composition of plants was checked by GC/MS. The amount and type of chemical compounds was achieved by comparing the data from GC/MS with information for libraries. According to the results, 86 kinds of chemical compounds found in *M.anisodan* extract. Furfural, steroids, vitamin B and flavonoids are the main compounds of *M.anisodan*. previous studies have reported that some compounds such as diterpenes, sterol, derivatives of caffeic acid and flavonoids exists in this genus (Meyre and Cechinel, 2010). 46 kinds of chemical compounds found in methanol extract of *E.khuzestanium*. there are mucilage, fatty acids, flavonoid and diterpenes in flower of *E.khuzestanium*. Delorme et al (1977) was reported that E.amoenum have anthocyanins (13%), flavonoids (0.15%) and a small amount of alkaloids. Javadzade (1995) was reported that *Borago officinalis* have mucilage, tannins, Na, Ca and K. None of the plant extracts had antifungal effect and No halo was observed.

4. DISCUSSION

In previous studies have not been reported antifungal effect of these two plants. Essential oil of M.vulgar had antifungal effect on *Botrytis cinerea* and poor antifungal effect on *Fuzarium solani* ،*Penicillium digitatum* and *Aspergillus niger* (zeid etal, 2011). Research shows that the flavone, tannins and alkaloids have antibacterial and

antifungal effects(Cowan, 1999; Dixon and Steele, 1999). the absence of anti-fungal properties of these plants can be due to not being pure active compounds or low percentage of active compounds.

CONCLUSION

To reduce the use of pesticides, the researchers have sought to achieve more natural and environmentally friendly ingredients. Despite of efficacy, compounds are quested that break down quickly and leave residues in food less**.** In this way, the exact chemical structure and inhibitory effects of metabolites extracted from various plants should be identified and studied. Also studying plant extracts on other fungi is necessary because there are possibility that extracts without antifungal activity in this study, have antifungal effects on other fungi.

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