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PROMISING ANTIFUNGAL EFFECT OF SOME FOLKLORIC MEDICINAL PLANTS COLLECTED FROM EL- HAMMAM HABITAT, EGYPT AGAINST DANGEROUS PATHOGENIC AND TOXINOGENIC FUNGI

Sawsan Abd-Ellatif[1], Salwa M. Abdel Rahman[2] and Sahar F. Deraz[3]
[1]Department of Bioprocess Development, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research and Applied Technology, Research Zone, Borg Al-Arab, Alexandria, Egypt
[2]Department of Botany, Faculty of Science, Alexandria University, Moharram Bey, Alexandria, Egypt
[3]Department of Protein Research, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research and Applied Technology, Research Zone, Borg Al-Arab, Alexandria, Egypt
E-Mail: sahar_deraz@hotmail.com

ABSTRACT

Increasing evidence of fungicide-resistant toxinogenic and pathogenic fungal species is obvious. Looking for new possibilities of antifungal treatment or sources of antifungal substances is a major problem. Some medicinal plants exert strong antifungal properties and could be conveniently used as a promising alternative source for presently problematic antifungal treatment in many areas with respect to their natural origin. In this study, antifungal activities of *Mesembryanthemum crystallinum* (Aizoaceae); *Blackiella aellen*, *Arthrocnemon glaucum* and *Atriplex halimus* (Chenopodiaceae), *Thymelaea hirsute* (Thymelaeaceae), *Carduus getulus*, *Atractylis carduus* and *Echinops spinosissimus* (Compositae), *Nicotiana glauca* (Solanaceae), *Alhagi maurorum* (Fabaceae) species were investigated. The fungal effects of these plants were tested by agar tube dilution method using *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus flavus*, *Alternaria alternate*, *Rhizoctnia solani*, *Pythium ultimum*, *Bipolaris oryzae*, *Rhizopus*, *Chetomium* and *Mucor*. For 10 plant species, the possibility of using them as natural fungicides was indicated. The extracts showed significant activity against most target fungal species. The most sensitive target funguses were the toxinogenic and human pathogenic species *Fusarium solani* and *Bipolaris oryzae* plant methanol and hexane extracts, respectively. The overall results provide promising base line information for the potential use of the crude extracts of tested plants in the treatment of fungal infections. An attempt has been made to highlight the promising plant species for further investigation as leads for new drug development.

Keywords: medicinal plants, antifungal activity, fungi, biological control, hexane extracts, methanol extracts, nutraceuticals.

INTRODUCTION

Human and animal fungal infections pose serious medical and veterinary issues. In the past few decades, a worldwide increase in the incidence of fungal infections has been observed as well as a rise in the resistance of some species of fungus to different fungicidal used in medicinal practice. Fungal infection of plants represents significant losses of agricultural products. Up to now, more than 100,000 fungal species are considered as natural contaminants of agricultural and food products (Kacaniova, 2003; Sati and Joshi, 2011). Although there are several natural and synthetic products available to ameliorate fungal infections, the last two decades have witnessed a dramatic rise in the incidence of life threatening systemic fungal infections.

There is currently an increase in the numbers of immune compromised individuals due to advances in medical technology and a pan epidemic of HIV infections. With the rise in-at risk patients, the number of invasive fungal infections has dramatically increased in both developed and developing countries (Meena *et al*., 2009). The challenge has been to develop effective strategies for the treatment of candidiasis and other fungal diseases, considering the increase in opportunistic fungal infections in human immunodeficiency virus-positive patients and in others who are immuno compromised due to cancer chemotherapy and the indiscriminate use of antibiotics.

Most of green plants represent a reservoir of effective chemo-therapeutants and can provide valuable sources of natural drugs, natural pesticides and biofertilizers. In designing a search for novel prototype antifungals, it seems reasonable to assume that if new agents are to be found that have different structures and different activities from those in current use, sources other than the more traditional plant extracts must also be investigated. Therefore, it is quite logical that any recent search for new prototype antifungal products should also include a variety of plant part or extract. In particular, higher plants are a logical choice, chiefly because of their seemingly infinite variety of novel molecules, which are often referred to as secondary metabolites (Clark and Hufford, 1992). Antifungal agents are widely distributed among higher plants (Caceres *et al*., 1991), but only a few have been evaluated for their activity against human, animal and plant pathogenic fungi.

The presence of antifungal compounds, in higher plants, has long been recognized as an important factor in disease resistance. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Siva *et al*., 2008). In addition, plant extracts might have inhibitors to enzymes from the invading pathogens, and the effects of different phenolic compounds on the germination and growth of

many fungal pathogens have been reported (Amadi *et al*., 2010).

Plant extracts and their essential oils show antifungal activity against a wide rang of fungi (Cowan, 1999; Kurita *et al*., 1981; Grane and Ahmed, 1988; Abd-Alla *et al*., 2001; Wilson *et al*., 1997). Several authors studied the effect of different plant extracts on the growth of fungi: *Cymbopogon proximus* against the toxigenic fungi *Fusarium erticillioides* and *Aspergillus flavus* (El-Assiuty *et al*., 2006); *Allium sativum*, *Cymogopogon proximus*, *Carum carvi*, *Azadirchia indica* (neem) and *Eugenia caryophyllus* against *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea* and *Rhizoctonia solani* (Aba AlKhail, 2005); and *Aristea ecklonnii* and *Agapathus inapertus* against *Botrytis cinerea*, *Fusarium oxysporum*, *Rhizoctonia solani* (Pretorius *et al*., 2002).

Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Mahesh and Satish, 2008). Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antifungal agents, in this study, a systematic investigation was undertaken to screen the thimble and extracted successively with methanol and hexane local flora for antifungal activity from *Mesembryanthemum crystallinum*, *Blackiella aellen*, *Arthrocnemon glaucum*, *A triplex halimus*, *Thymelaea hirsute*, *Carduus getulus*, *Nicotiana glauca*, *Alhagi maurorum*, *Atractylis carduus*, *Echinops spinosissimus.*

MATERIAL AND METHODS

Plant collection

The aerial parts of ten selected plants (Table-1) were collected from El- Hammam region in the Western Mediterranean coastal part of Egypt during spring season of 2009. Plant species were collected and identified at faculty of Science, Alexandria University and investigated.

Table-1. List of plant species screened for antifungal activity.

Extract No.	Plant Names		Family
	Potenical name	**Common name**	
1	*Mesembryanthemum crystallinum*	Crystalline ice plant Slender-leaf ice plant	*Aizoaceae*
2	*Blackiella conduplicata (F. Muell.) aellen*	*Blackiella aellen*	*Chenopodiaceae*
3	*Arthrocnemon glaucum*	Shinaan, oshnan, Hatab ar ad, Khriyet, Khreiza	*Chenopodiaceae*
4	*Atriplex halimus*	*Sea orach*	*Chenopodiaceae*
5	*Thymelaea hirsute*	Mithnaan *gy Sparrow-*	*Thymelaeaceae*
6	*Carduus getulus*	Hoshroof	*Compositae*
7	*Nicotiana glauca*	Massas	*Solanaceae*
8	*Alhagi maurorum*	Aqool camel's thorn, Persian Manna plant	*Fabaceae*
9	*Atractylis carduus*	Shawk El-Gamal	*Compositae*
10	*Echinops spinosissimus*	Kadaad	*Compositae*

Preparation of plant extracts

After collection, the fresh aerial parts were cleaned with water, air-dried and coarsely powdered using a mortar and pestle (and were further reduced to powder using electric blender). To obtain fractions with different polarities, each of powdered air-dried plant material was extracted with hexan and methanol (1L each). Extraction with each solvent was done during two weeks period with occasional shaking. The two extracts for each sample were collected and concentrated to dryness using rotary evaporator at 40^{o}C. The dried extracts were weighed and then stored in air tight container and kept at 4^{o}C until analysis.

Microbial samples

One yeast and ten fungal pathogens were locally isolated and identified by classical methods (Menghini *et al*., 1987). These fungi represent different morphological forms of fungi namely yeast (*Candida albicans*) and moulds (*Fusarium solani*, *Fusarium oxysporum*, *Aspergillus flavus*, *Alternaria alternate*, *Rhizoctnia solani*, *Pythium ultimum*, *Bipolaris oryzae*, *Rhizopus*, *Chetomium*, *Mucor*). These are most common and most important disease causing fungi of animals, plant and human being.

Preparation and standardization of inoculums

The fungal strains were maintained on sabouraud dextrose agar medium, respectively. All strains were kept

at 10°C, sub- cultured and checked for purity every 4-5 weeks. For mycelial fungal strains, to sabouraud dextrose agar (SDA) tubes containing 4 ml, inoculated with 4 mm diameter piece removed from a seven-day-old culture of each fungus. An agar surface streak is employed for non-mycelial growth (*Candida*).

Antifungal activity test

For antifungal bioassay, agar tube dilution method was used (Berhge and Vlietinck, 1991). Twenty three mg of crude extract was dissolved in 1 ml sterile DMSO serving as a stock solution. Then transferred 4 ml sabouraud dextrose agar (SDA) growth media in each screw capped tube, under sterile condition and autoclaved at 121ºC for 15 min. These tubes were allowed to cool to 50ºC and 61 µl of each plant extract stock solutions were loaded in the SDA Tubes were then allowed to solidify in slanting position at room temperature. Other media supplemented with DMSO and reference antifungal drugs (miconazol) used as a negative and positive control, respectively. Then each glass tube was inoculated with 4 mm diameter piece of inoculums removed from 7 days old culture of fungus. In case of non- mycelial growth an agar streak was employed. All tubes were incubated at 28±1ºC for 7 to 10 days. Cultures were examined twice weekly during incubation. Linear growth was measured using ruler and recorded in mm and growth inhibition was calculated with reference to the negative control through the following formula:

$$\% \text{ Inhibition} = 100 - \left\{ \frac{\text{Lineargrowthintest(mm)}}{\text{Linear growth in control (mm)}} \times 100 \right\}$$

The antifungal activity of the studied plants extracts was evaluated according to the following criteria: inhibition % 30-40 = low activity, 50-60 = moderate activity, 60-70 = good activity, above 70 = significant activity.

RESULTS

This article describes the antimicrobial activities of some regional and 'forgotten' medicinal plants. A total of 20 extracts from 10 different plants species belonging to 6 families were tested. In Table-1, the botanical name, common name and families of the selected plant species are shown.

Antifungal activity of extracts

Antifungal activities against selected fungal microorganisms were recorded (Tables 2 to 4). In this study, the plant materials were extracted with two different solvents (methanol and hexane). Methanol was the quantitatively the best extractant, extracting a greater quantity of plant material than hexane. The results of the investigations show that the two extracts (methanol and hexane) from different tested plants possess antifungal activities against most of the tested organisms at a concentration of 23 mg/ml. The two extracts compared favorably with the standard antibiotic miconazol.

Methanol extracts

All the methanol extracts showed strong antifungal activity in regards to at least three fungal strains (Table-2).

Table-2. Antifungal activity of methanol extract of different medicinal plants against some pathogenic fungi at 23 mg/ml concentration.

Plant name	**Inhibition % of selected fungal pathogenic**									
	Aspergillus flavus	*Alternaria alternate*	*Fusarium oxysporum*	*Fusarium solani*	*Bipolaris oryzae*	*Rhizoctnia solani*	*Pythium ultimum.*	*Chetomium*	*Rhizopus*	*Mucor*
Mesembryanthemum crystallinum	46.7	60.0	89.3	94.6	90.0	21.5	00.0	41.2	31.6	58.4
Blackiella conduplicata	34.7	00.0	53.3	94.6	87.5	25.8	43.4	27.1	17.9	28.6
Arthrocnemon glaucum	37.4	92.0	62.0	94.6	66.7	00.0	00.0	00.0	52.2	67.2
Atriplex halimus	00.0	33.4	28.6	91.4	46.7	00.0	26.7	38.9	11.0	17.2
Thymelaea hirsute	24.0	86.7	58.4	92.4	91.7	15.6	00.0	94.2	93.2	21.5
Carduus getulus	56.0	77.4	88.1	67.4	93.5	65.8	00.0	16.8	59.0	70.3
Nicotiana glauca	6.7	93.3	00.0	94.6	91.7	00.0	00.0	23.6	19.2	00.0
Alhagi maurorum	33.4	89.4	89.3	94.6	91.7	00.0	00.0	59.0	00.0	94.1
Atractylis carduus	56	93.4	94.1	94.6	85	92.9	26.7	70.6	66.3	46.6
Echinops pinosissimus	72.0	88.0	94.1	94.6	86.7	00.0	00.0	64.8	52.1	67.9
Positive control*	5.9	50.0	50.0	100	20.0	31.3	00.0	100	37.5	11.2

*Antifungal drug (miconazol) serving as positive control, DMSO serving as negative control

Note: Zone of inhibition was 0.00 in aqueous control in all the concentrations against all the test bacteria

Of the different methanol plant extracts used in the investigation, the most active extracts against tested fungal strains were *Atractylis carduus* (100%); *Carduus getulus*, *Echinops spinosissimus*, *Mesembryanthemum crystallinum* (80%); *Alhagi maurorum*, *Arthrocnemon glaucum* (70%) and *Thymelaea hirsute* (60%). They were able to inhibit 10, 8, 7 and 6 out of 10 fungal strains of interest, respectively. On the other hand the least active methanol extracts were *Nicotiana glauca* (30%); *Atriplex halimus* (40%) and *Blackiella conduplicata* (F. Muell.) Aellen (50%). *Pythium ultimum* were found to be the most resistance fungal strain to most of the tested methanol extracts. On the contrary, *Fusarium solani* (Figure-1) and *Bipolaris oryzae* followed by *Alternaria alternate* and *Fusarium oxysporum* (Figure-2 (a) and (b)) were the most susceptible fungi to almost the entire methanol plant extracts tested.

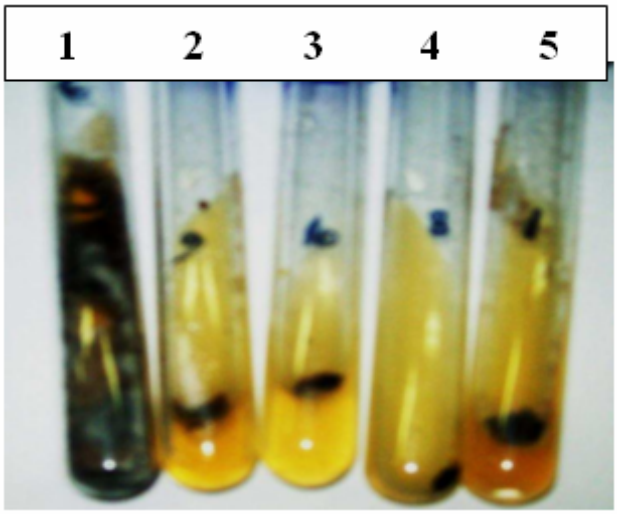

Figure-1. Photograph ‘‘standards’’ of *Fusarium solani* growth controls using different methanol extracts. Tube 1, negative control; tube 2, *Atractylis carduus*; tube 3, *Echinops spinosissimus*; tube 4, *Alhagi maurorum*; tube 5, *Mesembryanthemum crystallinum*.

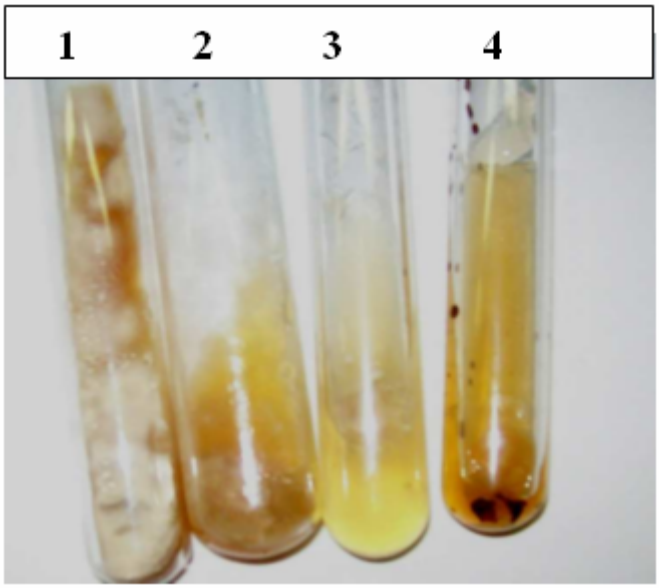

Figure-2 (A). Photograph ‘‘standards’’ of *Fusarium oxysporum* growth controls using different methanol extracts. Tube 1, negative control; tube 2, *Carduus getulus*; tube 3, *Atractylis carduus*; tube 4, *Echinops spinosissimus*.

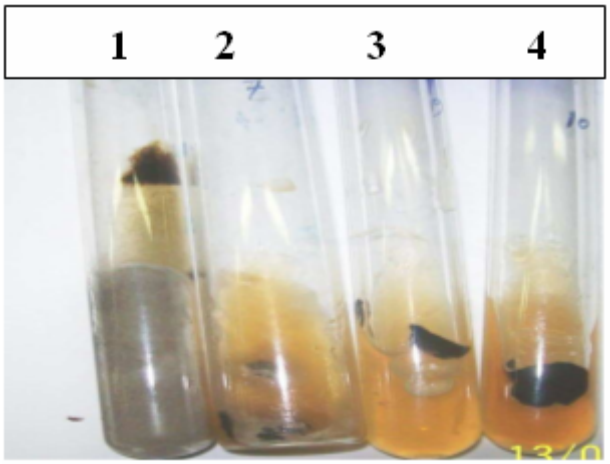

Figure-2 (B). Photograph ''standards'' of *Alternaria alternate* growth controls using different methanol extracts. Tube 1, negative control; tube 2, *Nicotiana glauca*; tube 3, *Atractylis carduus*; tube 4, *Alhagi maurorum*.

Hexane extracts

Among the aerial tissues of hexane extracted plant species tested, only the extract of fresh aerial tissue of *Mesembryanthemum crystallinum*, *Blackiella conduplicata* (F. Muell.) *Aellen*, *Arthrocnemon glaucum*, *Thymelaea hirsute*, *Carduus getulus* and *Nicotiana glauca* showed larger yield than other active extracts tested. Among the six hexane extracts tested, all the test fungi were highly sensitive to at least one of the tested extracts (Table-3).

Table-3. Antifungal activity of hexane extract of different medicinal plants against some pathogenic fungi at 23 mg/ml concentration.

Plant name	**Inhibition % of selected fungal pathogenic**									
	Aspergillus flavus	***Alternaria alternate***	***Fusarium oxysporum***	***Fusarium solani***	***Bipolaris oryzae***	***Rhizoctnia solani***	***Pythium ultimum.***	***Chetomium***	***Rhizopus***	***Mucor***
Mesembryanthemum crystallinum	58.9	11.8	94.5	17.7	100	93.8	87.5	44.5	68.8	75.6
Blackiella conduplicata	82.4	17.7	94.5	100	26.7	93.8	62.5	50.0	8.8	ND
Arthrocnemon glaucum	64.8	64.8	27.3	ND	66.7	62.5	43.8	33.4	43.8	83.4
Thymelaea hirsute	29.5	53.0	00.0	70.6	73.4	31.3	ND	22.3	87.5	16.7
Carduus getulus	29.5	29.5	44.5	41.2	73.4	25.0	37.5	33.4	18.8	44.5
Nicotiana glauca	100	44.8	77.8	74.2	80.0	56.3	68.8	11.2	81.3	38.9
Positive control*	5.9	50.0	50.0	100	20.0	31.3	00.0	100	37.5	11.2

*Antifungal drug (miconazol) serving as positive control, DMSO serving as negative control
Note: Zone of inhibition was 0.00 in aqueous control in all the concentrations against all the test bacteria

On contrast to methanol extract, *Nicotiana glauca* (90%) was the most highly active hexane extract against 9 out of 10 fungal strains of interest, followed by *Mesembryanthemum crystallinum* and *Arthrocnemon glaucum* (80%). None of the hexane extracts was able to highly inhibit the fungal pathogen *Chetomium*. Whereas *Mesembryanthemum crystallinum*, *Blackiella conduplicata* (F. Muell.) *Aellen* and *Nicotiana glauca* were able to entirely inhibit (100%) the growth of *Bipolaris oryzae*, *Fusarium solani* and *Aspergillus flavus*, respectively.

The hexane extracts generally showed stronger activity against the test fungal strains compared to the positive control (antibiotic miconazol) except for *Chetomium* and *Fusarium solani*.

For non- mycelial fungi, results in the present study relieved that most of the tested extracts posse's potential activity against *Candida albicans*. In methanol extracts (6 mg/ml), the maximum zone of inhibition was observed with *Carduus getulus* (20 mm) followed by *Echinops spinosissimus* (13mm), *Blackiella conduplicata* (12 mm) and *Atriplex halimus* (11mm). In some cases, increasing the concentration of the tested extracts (23 mg/ml) of the same plant had higher potent activity, such results were observed with extracts of *Mesembryanthemum crystallinum* (22mm), *Arthrocnemon glaucum* (23mm), *Nicotiana glauca*

(25mm) and *Atractylis carduus* (20mm). However, little or no activity was observed from the same extracts at 6mg/ml (Table-4 and Figure-3). Anticandiada activity of methanol extracts showed significant activity when compared with the hexane extracts at same concentration of 6 mg/ml of the different hexane plant extracts used in the investigation, only extracts of *Blackiella conduplicata*, *Atriplex halimus*, *Alhagi maurorum* and *Nicotiana glauca* showed activity against *Candida albicans*.

Table-4. Anticandidiasis activity of solvent extracts of different medicinal plants.

Plant name	Zone of inhibition (mm) of *Candida albicans*		
	Methanol extract 6 mg/ml	Methanol extract 23 mg/ml	Hexane extract 6 g/ml
Mesembryanthemum crystallinum	8	22	0
Blackiella conduplicata	12	15	10
Arthrocnemon glaucum	9	23	0
Atriplex halimus	11	10	19
Thymelaea hirsute	9	8	0
Carduus getulus	20	23	0
Nicotiana glauca	7	25	14
Alhagi maurorum	10	10	8
Atractylis carduus	7	20	0
Echinops spinosissimus	13	18	0
Positive control [1]	22	30	22

[1] Antibacterial drug (Imipenum) serving as positive control, DMSO serving as negative control
Note: Zone of inhibition was 0.00 in negative control in all the concentrations against all the test bacteria

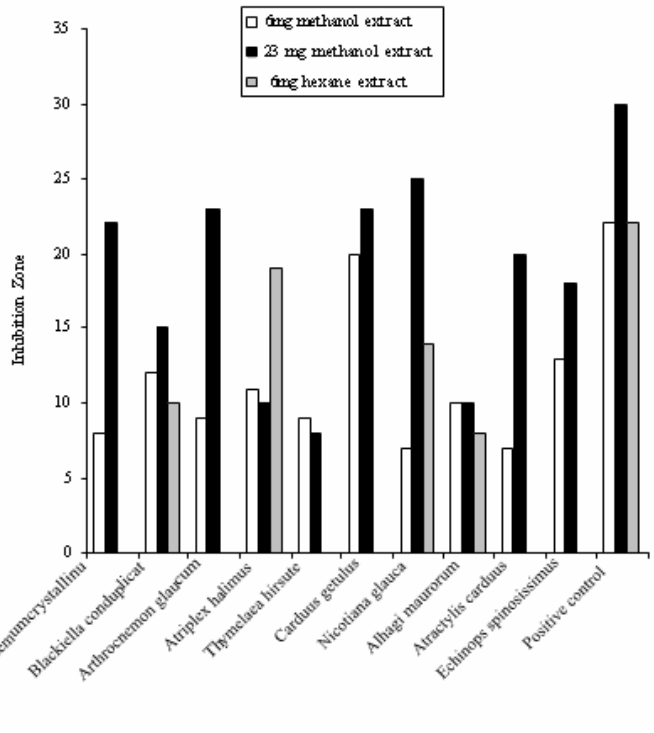

Figure-3. Anticandidiasis activity of methanol and hexane extracts of different medicinal plants.

DISCUSSIONS

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. In an approach toward the development of ecofriendly antifungal compounds for controlling major fungal diseases of methanol and hexane extracts of 10 plants belonging to 6 different families collected from El- Hammam region in the Western Mediterranean coastal (Egypt) were tested against the fungal pathogens. The first step towards this goal is the *in vitro* antifungal activity assay (Tona *et al*., 1998). The method used in this study is the dilution-tube-susceptibility test, which is an effective method to evaluate the antifungal of any filling material or solution (Baron *et al*., 1994). This method allows direct contact in the solution between fungal cells and the tested material. In addition, such method is considered appropriate when evaluating antifungal activity of materials, which has a low solubility and diffusibility (Torabinejad *et al*., 1995).

Many reports are available on the antiviral, antibacterial, antifungal, have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the exploitation of antifungal property of plants for developing commercial formulations for applications in crop protection.

Different types of *Fusarium* spp. are the causal organisms of soil born disease of plants and sometimes they may cause seed born disease also. They cause many wilting diseases in several crops. *F. oxysporum* also causes the wilting disease of lentil, tomato and banana. *Alternaria alternata* has been recorded causing leaf spot and other diseases on over 380 host species. It is opportunistic pathogen on numerous hosts causing leaf spots, rots and blights on many plant parts. It can also cause upper respiratory tract infections and asthma in people with sensitivity (Wiest *et al*., 1987). *Bipolaris oryzae* is classified in the subdivision Deuteromycotina (imperfect fungi) and family Dematiaceae and is the causal agent of brown spot disease of rice. Brown spot is one of the important rice diseases in the world. In Egypt, the disease comes in the second rank after blast disease (Shabana *et al*., 2008). *Rhizoctonia solani* is a plant pathogenic fungus with a wide host range and worldwide distribution. *Rhizoctonia solani* and *Pythium ultimum* are the cause of the condition known as damping off, which is a cause of death of seedlings in agriculture (Hillocks, 1992; Franc *et al*., 2001). Furthermore, some of the previous mentioned fungal pathogenesis has been frequently reported as etiologic agents of opportunistic infections in humans. Onychomycosis is defined as an infection of nails caused by fungi. The most important non-dermatophyte moulds causing onychomycosis are said to be *Alternaria alternata*, *Aspergillus* spp., *Fusarium* spp., *Nattrassia mangiferae* (Godoy *et al*., 2004). Biological control had attained importance in modern agriculture to curtail the hazards of intensive use of chemicals for pest and disease control (Tuber and Baker, 1988). Accordingly, the observed efficacy of different plant extracts tested in the present study explores the possibilities of controlling fungal pathogenesis by using plant extracts and highlights on results encouraging the possible application in agriculture after field investigations.

The result obtained in this study has provided a scientific support for the claimed ethnomedical uses of plant extracts of *Mesembryanthemum crystallinum*, *Blackiella aellen*, *Arthrocnemon glaucum*, *Atriplex halimus*, *Thymelaea hirsute*, *Carduus getulus*, *Nicotiana glauca*, *Alhagi maurorum*, *Atractylis carduus* and *Echinops spinosissimus* in the treatment of fungal diseases and suggest its potential as antifungal agent that could be useful in the current search of antimycotic agent from plants.

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