

# ANTIFUNGAL AGENTS FROM CERTAIN PLANTS

#### Presented By

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## Approval Sheet

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# رسالة دكتوراه

أسم الطالبة: أمل أحمد ابراهيم مكاوى (ماجستير عل وم ميكروبيولوجي)

عنوان الرسالة: دراسة المضادات الفطرية من بعض النباتات

الدرجة العلمية: الدكتوراه الفلسفة في العلوم ( الميكروبيولوجي )

#### تحت أشراف

# الاستاذ الدكتور / غبدالرزاق غبدالرحمن ابوسعده

رئيس قسم النبات و الميكر وبيولوجى بكلية العلوم رئيس المركز الاقليمي للفطريات و تطبيقاتها جامعة الأزهر

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الدرجة العلمية: الدكتوراه الفلسفة في العلوم (الميكروبيولوجي)

القسم التابع له: النبات

الكلية : كلية البنات للاداب و العلوم والتربية

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# شكر

اتوجه اولاً الى الله عز و جل بخالص الشكر و الدعاء على توفيقه لى كما اتوجه بخالص تحياتي و شكرى الى :

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عنوان الرسالة: دراسة المضادات الفطرية من بعض النباتات

الدرجة العلمية :الدكتوراه الفلسفة في العلوم ( الميكروبيولوجي )

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Introduction -1-

## **Introduction**

Invasive fungal infections can be classified as superficial and systemic mycoses, based on which areas of the body are primary affected. Systemic fungal infections have continued to increase in incidence during the past 20 years and are now significant important causes of high morbidity and mortality of hospital patients (Lee et al., 1998). This is particularly true in patients with haematological malignancies undergoing induction or consolidation chemotherapy, in immuno-suppressed organ transplant recipients, and in patients with acquired immunodeficiency secondary to infection by human immunodeficiency viruses. These infections also occur in some iatrogenic or nosocomial clinical settings (Andriole, 1998 and Groll et al., 1998).

Autopsy data indicate that more than half of the patients who die with malignancies are infected with *Candida* sp., approximately one-third with *Aspergillus* sp., and increasing numbers with *Cryptococcus* sp. or other fungi such as *Fusarium* sp. (Walsh, 1992 and Andriole, 1998). However, only small numbers of drugs are available for the treatment of life-threatening fungal infections. The exploitations of plants for medicinal uses have a long and honorable history. For a long time, the plant was considered to be "Natural's green pharmacy" which has recently played an important role in the treatment of some infectious diseases in humans and provided drugs for the maintenance of a good health (Bammert and Fostel, 2000). During the last few decades, there has been an interest in plants as a source of medicine.

There were a number of reasons for this interest: Firstly, there is a belief in developing countries that their economical drug problems can be solved with scientific exploitation of medicinal plants. Secondly, the whole world is returning to the green nature, believing that herbal remedies are safer and less damaging to the human body than synthetic drugs. Thirdly, drug plants supply more efficiently for some conditions where the synthetic drugs have a little to offer (**Braun et al., 2000**).

Climate throughout most of the year most of Egypt is hot and dry. Alexandria and the Mediterranean coastal communities experience milder weather while the heat in Cairo and other areas is fierce with temperatures rising to as high as 50°C in some parts of the country. Heavy winter rains fall along the Mediterranean coast but are less frequent in Cairo and in the interior. During the winter months (from November to February), however, temperatures can fall to freezing. The spring in Egypt is generally mild, a hot and bitter wind that brings blinding sand and dust storms and heralds the coming of summer. The Nile Delta and the Nile River Valley have a rich variety of trees, some indigenous, some imported - including the *Tamarix*,

Bassia, Plantago, Acacia, Mimosa, Jacaranda, Cypress and Sycamore as well as a wide variety of fruit trees from Citrus, Fig to Banana (Baser, 1992).

The flora in Egypt differ drastically between the Nile River Valley and the vast desert regions. Egyptian flora and El-Koran El-Kareem (Islamic Holy Book) mentioned plants had been used successfully by herbalists in many countries in Africa, for the treatment of various human diseases. There are a large number of these plants attributed with antimicrobial and anthelmintic actions. Also, some natural products of these plants are generally used as antifungal (especially as antidermatophytes) (El-Shanawany, 1993).

Traditional Egyptian plant flora was previously not accepted by many modern microbiologist scientists. However, in recent years, scientific studies have begun to explain some of the curative phenomena associated with traditional herbal remedies. A major reason for the selection of a wild plant for this study is the claims of the "traditional healers" for therapeutic usefulness of this plant. The medicinal value of a drug plant lies mainly in its active constituents like terpens, alkaloids, flavenoids, steroids, tannins, volatile oils, cardiac glycosides, saponins and/or resin, mucilage and others (Nwosu and Okafor, 1995).

Fungal infections in human are a major problem in immuno-compromised patients. The recent expansion of antifungal drug research has occurred because there is a critical need for new antifungal agents to treat these life-threatening invasive infections (**Hube** et al., 2001). The overview of the development of antifungal therapy, which is provided herein, reflects the increased interest in this very special area of infectious diseases (**Backer** et al., 2001).

Although we have newer, less toxic, antifungal agents that are available for clinical use, their clinical efficacy in some invasive fungal infections, such as aspergillosis and fusariosis, is not optimal. Thus, intense efforts in new effective antifungal drug from natural sources discovery are still needed to develop more promising and effective antifungal agents for use in the clinical arena. It becomes crucial to activate such field of research particularly with the approval of agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS)(Lesuisse et al., 2002).

So that, the current study aims to evaluate *in vitro* the value of natural desert plants and plants mentioned in The Holy Koran encloses biological activities as antifungal agents against certain pathogenic fungi, effect on morphological and ultrastructures features of certain fungi. Finally, chemical isolation, purification and identification of highly potent active compounds from plant extracts by NMR, IR and Mass spectrum.

# Review of Literature

# I- COMMON CHARACTERISTICS OF CERTAIN DESERT EGYPTIAN FLORA:

Arid and semi-regions account for about 30% of the total land surface of the earth and can significantly contribute to agricultural productions (Aly, 1998). In countries having large areas of deserts, like Egypt (Egypt is part of the Sahara "North African Desert", its area is about one million km² divided by the River Nile into a Western Desert of about 681,000 km² and an eastern part comprising the eastern Desert 223,000 km² and the Sinai peninsula 61,000 km²), the limited number of species, whose habitats are generally at risk, is a better reason for enumeration,, description and conservation. These plants produced secondary metabolites, to protect themselves from physical and biotic aggressions. These metabolites are the stuff of the medicinal uses of these plants (Boulos, 1999).

Pharmacological activities of several desert plants were investigated, several of flora plants used for its diuretic properties, in the treatment of dropsy, for fever and snake bits, antioedema agents (Aye et al., 1996 a), asthma and allergic rhino-conjunctivitis treatment (Schwartz et al., 1997), treatment of liver injury (Kalantari, 1998), anaemia, and as an anthelmintic, diabetes (Broadhurst et al., 2000), antiulcerogenic properties (Aye et al., 1996 b and Borrelli and Izzo, 2000), also anti-inflammatory effect was determined (Nunez et al., 1997 and Rodriguez et al., 2003), and antidiarrhoeal (Rao et al., 2003).

The main features that characterized the desert Egyptian flora used in this study were adapted from **Aly (1998) and Boulos (1999)**:

# 1- Mesembryanthemum crystallinum (Aizoaceae) DESCRIPTION (photo 1 "1[A,B]"):

**Plant:** Annual succulent herb covered by crystalline vesicles; stems 25-80 cm. procumbent, branched, often making mats.

**Leaf:** 3.0-8.0 cm in long and 2.0-5.0 cm in width; radical leaves opposite, spathulate, petiolate; the cauline alternate, obovate, undulate, shortly petiolate or sessile.

**Flower:** 1 cm in diameter, axillary, sub sessile; calyx-lobes broadly-ovate; staminodes numerous, longer than the calyx; capsule 1 cm

#### 2- Echium sericeum (Boraginaceae)

#### **DESCRIPTION** (photo 1 "2 [A,B]"):

**Plant:** Perennial herb, with procumbent stems woody root, hispid with black spots.

**Leaf:** Narrow-lanceolate, 6-10 cm in long and 5mm in width, alternate, acuminate, entire, sessile, grey hispid.

**Flower:** Perfect, irregular, purple-mauve in color, 2-3 cm in long, arranged in a terminal, simple or branched cymes or in scorpoid simple spikes. Sepals 5, linear to lanceolate; corolla funnel-shaped with 5 unequal rounded and spreading lobes, curved, with open throat and obliquely cut limb. Stamens 5, inserted below the middle of corolla tube, style bifid; ovary deeply 4- lobed.

Fruit: Ovoid nutlets, rugose.

#### 3- Paronychia argentea (Caryophyllaceae)

#### **DESCRIPTION** (photo 1 "3[A,B]"):

**Plant:** Annual or perennial herbs, small 5-30 cm; prostrate plants, branching from base into several spreading red pilose stems.

**Leaf:** Linear, oblong-lanceolate, mucronate, glabrous, flat, about 0.5-1.2 cm in long and 0.1-0.25 cm in width. Stipules (about 5 mm. broad) and bracts silvery, ovate to oblong-lanceolate, acuminate, scarious, greenish.

**Flower:** In heads hidden in silvery-scarious bracts or axillary and terminal clusters, 1-1.5 cm diameter. Bracts papery in texture pointed, at least twice the length of the flower and hence the plant of a more silvery aspect. Sepals (5) 1.5mm; broadly scarious-margined, globrous with straight hairs at the base and an apical awn equaling or shorter than the sepal; stamens 5, sometimes fewer, staminodes 5, filiform; styles 2 free or fused and 2-lobed at the apex, utricle 1.25 mm, ovoid-cylindrical, calyx of free sepals.

**Fruit:** Indehiscent. Fruits and flowers resembles tiny straggling mat, with a conspicuous overall silvery appearance, seeds lenticular or globose brown, glossy.

# 4- Atriplex portulacoides (Chenopodiaceae) DESCRIPTION (photo 2 "4"):

**Plant:** Robust shrub, monoecious, glabrescent; stems procumbent, branches ascending, striate; about 1-3 m high, erect. Branches numerous, white in color, lateral branching very large.

**Leaf:** Simple, 1- 5 cm in long, 0.3-1.2 cm in width, opposite, rather fleshy, grayish, short-petiolate, oblong-elliptic to linear-lanceolate, cuneate at the base, acute to obtuse or apiculate at the apex.

**Flower:** In clusters, forming terminal and axillary spikes, in paniculate inflorescences, staminate flowers with calyx and no bracts, the stamens as many as the segments, pistillate flowers usually without calyx and with 2