SURVEY OF SOME PLANT EXTRACTS AGAINST CERTAIN HUMAN FUNGAL PATHOGENS

RAGAB, A.M. AL-SAYED¹, EL- SHIEKH, H.H.², EMAN, M.A. EL-TAHER ³ AND BASMA. H. AMIN³

¹Plant & Microbiology Department, Faculty of Science,(Girls' Branch), Al- Azhar University ²Plant & Microbiology Department Faculty of Science,(Boys' Branch), Al- Azhar University ³The Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

Abstract

Antifungal agents (A.f.A.) play an important role in the treatment of human serious diseases. Therefore, A.f.A. from natural sources was preferred than that synthetic compounds. Methanol, methanol: water; (1:1), water, chloroform and benzene extracts of thirty two plant organs; sixteen desert and sixteen cultivated plant organs were assessed against forty six human fungal pathogenic isolates; twelve (yeast), two (yeast like fungi) and thirty two (filamentous fungi). From the five assessed extracts, chloroform extracts exhibit the most significant activity followed by benzene, methanol: water; (1:1) and methanol, while, water extracts exhibited the least activity. Chloroform extracts revealed the highest inhibitory effect against the mycelial growth of the human fungal isolates. *Thymus serpyllum L.* (desert plant) and *Anethum graveolens* (cultivated plant) were active against *Aspergillus tamarii* 1 and *Penicillium marneffei* isolated from eyes and blood, respectively. Scientifically, this study recommended the use of these plant extracts as a potent antifungal agent after their purification and identification.

Keywords: antifungal agents, plant extracts, human pathogenic fungi

Introduction:

People are at risk of fungal infections when they are taking high potent antibiotics for a long period of time because antibiotics damage not only the pathogenic bacteria, but healthy bacteria as well. This alters the balance of microorganisms in the mouth, vagina, intestines and other organs in the body, lead in an overgrowth of fungi of the individuals with weakened immune systems who are also at risk of developing fungal infections. This is the case of people with HIV/AIDS, people under steroid treatments, and people taking chemotherapy. People with diabetes also tend to develop fungal infections. Very young and very old people, also, are at risk. (Wikipedia, the free encyclopedia, 2010).

There is a need to develop a wider variety of antifungal agents that are more effective and less toxic, from cheaper and natural sources. Plant metabolites are considerably useful and economically essential. They contain active constituents, that are used in the treatment of many human diseases (Gayathri et al., 2011). The

plant extracts have been developed and proposed for use as antimicrobial agents (**Del**, *et al.*, **2000**). Many of the plant sources used in traditional medicine are readily available in rural areas and cheaper than chemotherapy (**Mann**, *et al.*, **2008**).

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 (Sawsan, *et al.*, 2011). There is currently an increase in the numbers of immune compromised individuals due to 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 is 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 (**Meena**, *et al.*, **2009**).

Some fungi are pathogenic to humans and cause a variety of diseases in normal and immunocompromised hosts. Fungi can infect any part of the human body including hairs and nails and depending upon their level of penetration is divided into four groups, superficial, cutaneous, subcutaneous and systemic/deep mycoses. Systemic mycoses are often fatal without treatment. The ability to maintain constant body temperature is the natural defence against pathogenic fungi. In the event of infection, accurate diagnosis and often the combination of different treatment modalities are required for a successful treatment (Neena and Bettina, 2011).

Most green plants represent a reservoir of effective chemo-therapeutic and can provide valuable sources of natural drugs. In designing a search for novel prototype antifungals, it seems reasonable to assume that, if new agents are to be found and have structures and activities differ 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 agents should also include a variety of plant organs or extract. In particular, higher plants are a logical choice, chiefly because of their seemingly infinite variety of novel agents, which are referred to as secondary metabolites (Clark and Hufford, 1992). Antifungal agents are widely distributed among higher plants, but only a few have been evaluated for their activity against human, animal and plant pathogenic fungi (Caceres, et al., 1991).

Plants have been classified as an essential source of medicinal agents for centuries and a huge number of novel drug components have been isolated from natural plant sources. Many of these plants and their extracts are used in traditional medicine. Medicinal plants play a key role in health care with about 80% of the world's populations relying on the use of traditional medicine which is predominantly based on plant types (Owolabi, et al., 2007).

Plant extracts and their essential oils exhibit antifungal activity against a wide range of fungi (Kurita, et al., 1981; Grane and Ahmed, 1988; Wilson, et al., 1997; Cowan, 1999 and Abd-Alla, et al., 2001). Several authors studied the effect of different plant extracts aganist 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).

Medicinal properties of aromatic plants and their extracts have been recognized since time immemorial. They are still used in medicine, food and cosmetic industry (Lahlou, 2004). Among these plant species, *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *Ocimun basilicum* (basil), *Lippia sidoides* (rosemary-pepper), *Plectranthus amboinicus* (mint), *Eucalypthus citriodora* (eucalyptus), *Syzygium cumini* (clove), *Allium sativum* (garlic), *Melaleuca alternifolia* (tea tree), *R. officinalis* (rosemary), *Z. officinalis* (ginger), *C. citratus* (lemongrass), *M. piperita* (peppermint) and *Cinnamomum zeilanicum* Blume (cinnamon) were described as broad-spectrum antimicrobial agents (Mueller and Mechler, 2005; Silva and Fernandes, 2010). Therapeutic effect of these plants can generally be attributed to their volatile fractions (essential oils) rather than their extracts (Lahlou, 2004).

Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Mahesh and Satish, 2008). Considering the vast potentiality of plants as sources of antimicrobial drugs with reference to antifungal agents, in this study, a systematic investigation was undertaken to screen the thimble and extracted successively with chloroform for antifungal activity from *Thymus serpyllum L*. and *Anethum graveolens*.

The objective of the present study is to assess antifungal activity and properties of various plant extracts (thirty two plants; sixteen desert and sixteen cultivated) against various human fungal pathogens.

Materials and Methods:

Isolation media:

The following media were used for the experimental studies.

Sabouraud Dextrose Agar (SDA):

According to **Atlas (1993),** the medium contains (g/l): dextrose, 20.0; peptone, 10.0; agar- agar, 20.0 and distilled water, 1.0 L. The pH of the medium was adjusted at $5.6 (\pm 0.2)$ and autoclaved at 121° C for 20 min. at 1.5 atmospheric pressure.

Taplin (1965) revealed that, the addition of 40 mg gentamicin sulfate/liter, to suppress chloramphenicol-resistant bacteria, which are occasionally present.

Sabouraud Dextrose Agar (SDA) with Cycloheximide:

The medium contained the same previous components with the addition of 0.4 g cycloheximide; for the isolation of dermatophytes from clinical samples **Dewitte-Orr**, *et al.* (2005).

Leeming & Notman Agar (LNA):

This medium is specified for the isolation and maintenance of *Malassezia* sp. *Malassezia* sp. is inoculated using fresh medium upon receipt with incubation period 3 weeks. It consists of (g/l): Peptone, 10.0; glucose, 10.0; yeast extract, 2.0; ox bile, 8.0; glycerol, 10.0; glycerol monostearate, 0.5; tween 60, 5.0; olive oil, 20.0; agar, 15.0 and distilled water, 1.0 L. The medium was autoclaved at 121°C for 20 min. under 1.5 atmospheric pressure (**Takamasa** *et al.*, 2007)

Isolation of human pathogenic fungi:

Collection and purification of samples:

Human samples from skin, sputum, nail, hair, ear, eye, blood, urine and vagina were collected (in summer and autumn) from 100 patients. The isolates were isolated using sabouraud dextrose gar (SDA), sabouraud dextrose gar (SDA) with cycloheximide and Leeming & Notman agar (LNA) media. Then, stored in slants for further investigations.

Morphological, examination and identification studies of human fungal isolates:

The morphological features of all human fungal isolates were investigated and subjected to the direct microscopic examination at **The Regional Center for Mycology and Biotechnology (RCMB)**, **Al- Azhar University** by using Atlas of clinical fungi **(De Hoog, et al., 2000)** and Peniciliium species were identified by using A laboratory guide to common Peniciliium species **(Pitt, 1991)**.

The yeast isolates were identified by (VITEK 2 system; Intuitive, icon-driven software in a familiar Windows® format) in **Armed Forces- Laboratory Center**.

Using **Image analysis system** [soft imaging system GmbH software (analysis pro ver.3.0) at **(RCMB)** was used for examining the alteration of morphological features of human fungal isolates. The cultures were examined by using light microscopy, after 5-7 days incubation for dermatophytes, 24 hours for yeast and/or yeast like fungi and 3-4 days for other fungi; using light microscopy. Scale bar = $10 \, \mu m$.

Collection of plant organs:

Thirty two plants from a medicinal plant shops; Cairo, Egypt. Sixteen desert and sixteen cultivated plants were bought. Leaves of Salvia officinalis, Centaurea cyanus, Cleome droserifolia, Acacia nilotica, Solenostemna arghel, Cymbopgon proximus, Thymus serpyllum, Lavendula pinnata, Mentha arvensis, Ammi majus, Deverra tortuosa, Cymbopogon citratus, Raphanus sativus, Thymus vulgaris, Mangifera indica, Citrus sinensis, Morus alba L., Citrullus vulgaris, Mentha peperita, Ammi visnaga, Citrus aurantium, Psidium guajava, Anethum graveolens, Trifolium alexandrinum, Raphanus sativus and Raphanus raphanistrum, fruit of Phsalis pruinosa, bulbs of Scilla maritime L. and Allium cepa, legume of Cassia angustifolia, flower of Matricaria chamomilla and Chrysanthemum morifolium, were used in this study.

Preparation of plant extracts:

The plant organs were thoroughly washed in running water and sterile distilled water and kept in shade to dry for one week. Dry materials were then ground finely to be powdered with the help of a blender. Then, fine particles were stored in clean container, for further analysis. Pure methanol, methanol: water was used as (1:1) proportions; sterile distilled water, chloroform and benzene were used as separated extraction solvents. Five hundreds milliliter of each solvent are added to 50 g of

powdery materials of each plant organ and homogenized for 20 min with the help of a homogenizer and then were allowed to stand for 1 hour. Extracts were passed through Whatman filter paper No.1 to remove the residual materials and were used as 100% pure extracts. Mixtures were then centrifuged at 6000 rpm for 10 min to obtain clear extracts. Solvents were allowed to evaporate completely to a solid form using a rotary evaporator. Complete the final extracts of each plant to 5 ml of each solvent (Shittul, et al., 2007; Sharma, et al., 2010 and Zaker and Mosallanejad, 2010).

Determination of antifungal potentialities of the selected plants:

Antifungal activities were expressed as the diameter of inhibition zones using hole - plate diffusion method; 0.5 cm diameter holes were cut in the agar using sterile cork borer in sabouraud dextrose agar sterile plates 9 cm, which had previously been seeded with the test fungal strain by using sterile cotton swabs; the swabs were streaked over the surface of the medium. The holes were filled by 200 μ l of each concentrated plant extract filtrate, plates were left in a cooled incubator at 4 °C for one hour for diffusion, then the plates were incubated for 24-48 hours for yeast and yeast-like fungi and 5-7 days for dermatophyte fungi. After the end of the incubation period, the inhibition zones were measured (Abde- Kader and Seddkey, 1995).

The inhibition zones were measured at two points along the diameter of the plate and the mean of these two measures calculated as the mean diameter of the colony. The inhibition zone in control sets was compared with that of various treatments (Anandaraj and Leela, 1996).

Results:

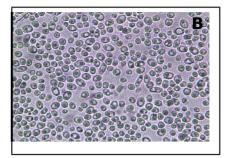
Identification of the fungal isolates:

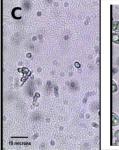
According to the identified and examined human fungal isolates; filamentous molds were identified at (RCMB) Al-Azhar University and the yeast species were identified at Armed Forces- Laboratory Center. (Table 1), (Fig. 1, 2, 3 and 4).

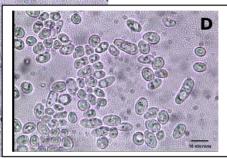
Table (1): Screening of human fungal isolates; Causative fungi, isolation sites, diseases, gender and age of patient.

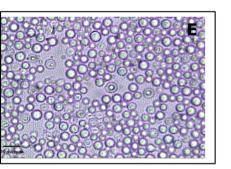
Isolate No.	Causative fungus	Isolation sites	Disease	Gender	Age
1	Candida albicans 1	Sputum	Chest infection	Female	35
2	Candida albicans 2	Ear	Otomycosis	Male	20
3	Candida albicans 3	Urine	Urinary tract infection	Female	37
4	Candida albicans 4	Vagina	Vulvovaginitis	Female	29
5	Candida famata 1	Vagina	Vulvovaginitis	Female	40
6	Candida famata 2	Blood	Candidiasis	Female	40
7	Candida kreusi	Eye	Keratitis	Male	51
8	Candida tropicalis 1	Vagina	Vulvovaginitis	Female	42
9	Candida tropicalis 2	Ear	Otomycosis	Male	38
10	Cryptococcus laurenti	Skin	Cryptococcosis	Male	36
11	Malassezia sympodialis	Skin	Skin infection	female	35
12	Rhodotorula glutinis	Blood	Blood infection	Male	68
13	Geotrichum candidum 1	Sputum	Giotrichosis	Female	17
14	Geotrichum candidum 2	Foot nail	Onychomycosis	Male	55
15	Aspergillus terreus 1	Ear	Otomycosis	Male	45
16	Aspergillus terreus 2	Eye	Keratitis	Male	66
17	Aspergillus niger 1	Ear	Otomycosis	Male	43
18	Aspergillus niger 2	Foot nail	Onychomycosis	Female	45
19	Aspergillus niger 3	Blood	Blood infection	Male	35
20	Aspergillus flavus 1	Hand nail	Onychomycosis	Female	40
21	Aspergillus flavus 2	Ear	Otomycosis	Male	28
22	Aspergillus flavus 3	Sputum	Aspergillosis	Male	35
23	Aspergillus flavus 4	Eye	Keratitis	Male	41
24	Aspergillus fumigatus 1	Urine	Aspergillosis	Male	38
25	Aspergillus fumigatus 2	Ear	Otomycosis	Male	30
26	Aspergillus fumigatus 3	Hand nail	Onychomycosis	Female	33
27	Aspergillus fumigatus 4	Eye	Keratitis	Male	53
28	Aspergillus tamarii 1	Eye	Keratitis	Male	55
29	Aspergillus tamarii 2	Blood	Aspergillosis	Male	25
30	Aspergillus nidulans	Foot nail	Onychomycosis	Female	35
31	Aspergillus ochraceous	Foot nail	Onychomycosis	Male	50
32	Penicillium duclanxii	Sputum	Chest infection	Female	40
33	Penicillium aurantiogriseum	Foot nail	Onychomycosis	Male	45
34	Penicillium chrysogenum	Eye	Keratitis	Male	60
35	Penicillium fellutanum	Foot nail	Onychomycosis	Male	53
36	Penicillium janczewskii	Ear	Otomycosis	Male	40
37	Penicillium marneffei	Blood	Blood infection	Male	35
38	Microsporum canis 1	Hair scalp	Tinea capitis	Boy	9
39	Microsporum canis 2	Skin	Tinea corporis	Female	32
40	Trichophyton mentagrophytes	Skin	Tinea corporis	Female	29
41	Trichophyton rubrum 1	Foot	Tinea Pedis	Male	53
42	Trichophyton rubrum 2	Groin	Tinea crusis	Female	35
43	Mucor hemalis	Ear	Otomycosis	Male	42
44	Abisidia corymbifera	Ear	Otomycosis	Male	50
45	Syncephlastrum racemosum	Foot nail	Onychomycosis	Male	40
46	Fusarium solani	Eye	Keratitis	Female	46



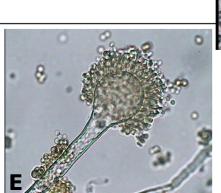






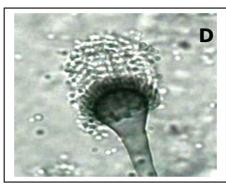


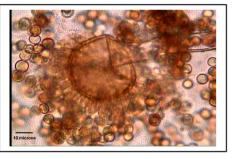




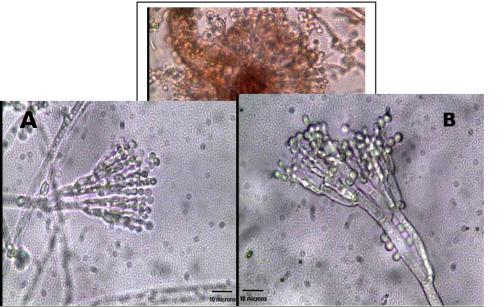


s (A), Candida ryptococcus laurenti (G). Scale bar =10









G

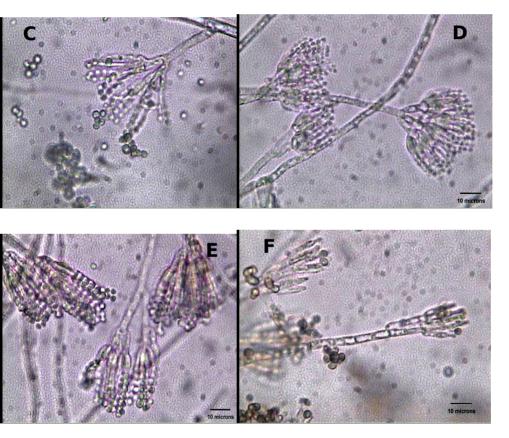
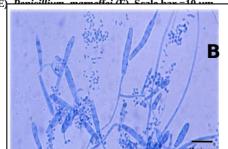


Fig. (3): Light microscopy micrograph of Pencillia; Penicillium duclanxii (A), Penicillium aurantiogriseum (B), Penicillium chrysogenum (C), Penicillium fellutanum (D),

**Penicillium ignazavachii (E) **Penicillium mamaffai (E) **Scala bax = 10 um





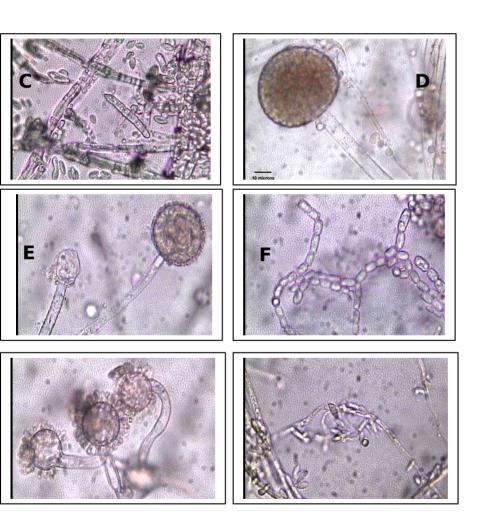


Fig. (4): Light microscopy micrograph of Microsporum canis (A), Trichophyton mentagrophytes (B), Trichophyton rubrum (C), Mucor hemalis (D), Abisidia corymbifera (E), Geotrichum candidum (F), Syncephlastrum racemosum (G) and Fusarium solani (H). Scale bar =10 μm .

Screening test of antifungal activity:

This study was conducted to detect the antifungal activities of thirty two botanical organ extracts using different solvent systems (methanol, methanol: water; (1:1), water, chloroform and benzene) against forty six human fungal pathogens isolated from different patient body sites. All the crude extracts had significant antifungal activities against the majority of the fungal isolates, but the of inhibition potentiality varied with the fungi with respect to the type of plant extract.

Among the solvents used for extraction, chloroform extracts revealed the most inhibitory effect against mycelial growth of the human fungal isolates, especially, *Thymus serpyllum L.* (desert plant) and *Anethum graveolens* (cultivated plant) against *Aspergillus tamarii* 1 isolated from the eye and *Penicillium marneffei* isolated from the blood.

The chloroform extract results were estimated as the follows: *Thymus serpyllum L.* exhibited good results against *Cryptococcus laurenti* (4.3 cm), *Aspergillus niger* 1 (4.5 cm), *Aspergillus flavus* 1 (4.6 cm), *Aspergillus tamarii* 1 (5.0 cm), *Aspergillus nidulans* (4.4 cm), *Aspergillus ochraceous* (4.3 cm), *Microsporum canis* 1 (4.5 cm) and *Abisidia corymbifera* (4.3 cm), while, *Penicillium marneffei* (6.0 cm). Also, *Anethum graveolens* gave the same activity against *Aspergillus tamarii* 1 (5.0 cm) and good results against *Penicillium duclanxii* (4.5 cm), *Penicillium aurantiogriseum* (4.5 cm), *Penicillium chrysogenum* (5.0 cm), *Penicillium fellutanum* (5.5 cm) and *Penicillium marneffei* (6.0 cm).

Also, the results revealed good effects of *Anethum graveolens* methanol extract against *Penicillium duclanxii* (5.5 cm), *Penicillium aurantiogriseum* (5.0 cm), *Penicillium chrysogenum* (5.5 cm), *Penicillium fellutanum* (4.5 cm) and *Penicillium janczewskii* (5.5 cm), while, *Thymus serpyllum* methanol extract gave the best result against *Microsporum canis* 1 (6.0 cm).

For methanol: water; (1:1) extracts, *Thymus serpyllum* exhibted good results against *Aspergillus terreus* **1** (4.5 cm) and *Mucor hemalis* (4.5 cm), while, *Solenostemna arghel, Artemisia inculta*, and *Phsalis pruinosa* gave good results (4.5 cm) against *Trichophyton rubrum* **1**.

For benzene extracts, *Thymus serpyllum* revealed good results against both *Mucor hemalis* (4.3 cm) and *Abisidia corymbifera* (5.0 cm).

However, water extracts had the least effect against most of the tested fungal isolates.

Discussion:

Plants generally produce many secondary metabolites which constitute an important source of many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (**Ibrahim**, 1997 and Ogundipe, 1998). Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management (**Aiyelagabe**, 2001 and Woldemichael, *et al.*, 2003). More natural antimicrobial agents leads the scientists to investigate the effectiveness of inhibitory compounds extracted from plants (**Abbas Abbas-Nasar S.M.**, **Halkman A.K.**, 2004).

Skin, hair, nail, and subcutaneous tissues in human are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytosis (Valeria, *et al.*, 1996 and Amer, *et al.*, 2006).

The antimicrobial activity of petroleum ether, ethanol, chloroform, n- hexane and water extracts of *Centella asiatica* has been studied against different fungi such as *A. niger* and *C. albicans* with inhibitory effect against all the tested microorganisms (Dash *et al.*, 2011).

All the crude extracts of *Dodonaea viscosa* have significant antifungal activity on most fungi, *Aspergillus niger*, *Aspergillus flavus*, *Paecilomyces varioti*, *Microsporum gypseum*, *and Trichophyton rubrum* causing skin diseases, but chloroform extract had maximum inhibition activity of 50-90.91% as compared to ethanol, methanol, ethylacetate and aqueous extracts have active inhibition activity in the range of 50-81.82% against tested dermatophytes (**Pirzada**, *et al.*, **2010**), which agrees with this study; chloroform extracts recorded the most inhibitory effect against the most of tested fungal isolates.

Loiy, et al., 2011 reported that the antimicrobial activities of crude chloroform, hexane and ethanol extracts of leaves, stems, fruits and seeds from *Citrullus lanatus var. citroides* (CL) against bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Proteus vulgaris*) and fungi (*Aspergillus nigar* and *Candida albicans*) were tested. The chloroform extract of the fruits exhibited the highest antibacterial activity, also, A. *nigar* was very sensitive to the chloroform extract of the seeds and the ethanolic extract of the leaves, these data agree with this study where chloroform extracts of the screened plants especially

Thymus serpyllum L and Anethum *graveolens* were active against the most human fungal pathogens.

On the other hand, oils extracts from leaves of *Anethum graveolens* and *Foeniculum vulgare* plants failed to exhibit antibacterial or antifungal activities against a variety of human <u>pathogens</u> (**Kazemi**, *et al.*, 2012), which contradicts with the results of study; are *Anethum graveolens* which gave better antifungal activities against the tested fungal isolates especially *Aspergillus tamarii* 1 isolated from the eyes and *Penicillium marneffei* isolated from blood.

Interestingly, the recent study agrees with **Pavel and Alcu**, 2008 who reported that the increase of fungal resistance to classical drugs and the treatment cost, and the fact that most available antifungal drugs have only fungistatic activity. *Thymus serpyllum* essential oil has proved its potential to be used as a topical antifungal agent against fungi that are pathogenic to humans. This essential oil showed an important activity against *Candida albicans* and *Candida glabratae*, which agrees with this study.

Adegoke, et al., 2010 reported that the antimicrobial activity of the aqueous, methanol and chloroform leaf extracts of Cissus multistriata were investigated against 8 bacterial and 2 fungal test organisms. They found that, the aqueous leaf extract had no activity against both bacterial and fungal test organisms. These results agree with the results in this study; most of methanol and chloroform leaf extracts gave the maximum inhibitory effects against the tested fungal isolates, since, aqueous leaf extracts exhibited the least effect against the most tested fungal isolates. Both methanol and chloroform leaf extracts inhibited all the test organisms, while, chloroform leaf extract revealed the highest inhibitory effect against *Escherichia coli*. These data matches with the results obtained in the present study in the case of chloroform results. The methanol leaf extract of *C. multistriata* revealed more antifungal activity compared with chloroform leaf extract, with Candida albicans being more susceptible than Aspergillus niger to both methanol and chloroform leaf extracts. These results contradict with the results obtained in this study; where Aspergillus niger being more susceptible than Candida albicans to both methanol and chloroform leaf extracts.

Ali and Abu Ghdeib (2002) reported that the aqueous extracts of 22 plant organs used in folkloric medicine in Palestine were investigated for their antifungal activity against *Microsporum canis* and *Trichophyton mentagrophytes*. Extracts of *Capparis spinosa* and *Juglans regia* completely prevented the growth of *M. canis*,

which agrees with the recent study when using *Ammi majus*. Also, *Pistacia lentiscus* prevented the growth of *T. mentagrophytes*, which agrees with the present data but with moderate efficiency when using *Allium cepa*.

Conclusion:

Therefore, this study revealed that chloroform extracts of the screened plant organs would be helpful in treating human fungal diseases especially *Thymus serpyllum* L (desert plant) and *Anethum graveolens* (cultivated plant) against *Aspergillus tamarii* 1 isolated from the eyes and *Penicillium marneffei* isolated from blood. Further studies are needed to isolate, characterize and elucidate the structure of the bioactive compounds of these plant organs for antifungal drugs formulation.

Acknowledgment: To Prof. Seham Ahmed Said (Head of the laboratory of National Institute of Urology and Nephrology, Cairo, Egypt); Dr. Hany Mohamed Magdy (Researcher at the Regional Center for Mycology and Biotechnology-RCMB, Al Azhar University; Prof. Ahmed Abd-Al Khalek Al Safty (Head of ENT department, Al Matarea Teaching Hospital); Prof. Amr Salah Al Dein (Head of Ophthalmology department, Al Kasr Al Eney Hospital) and Dr. Hoda Ragab Ahmed (Al Kasr Al Eney Hospital); Prof. Zeinab Al Khouly ((Head of Dermatology department, Al Matarea Teaching Hospital) and Dr. Yosra Abd-Al Galeil Abd-Al Halem (Dermatology department, Al Matarea Teaching Hospital); Prof. Mohamed Taha (Prof. of Microbiology and supervisor of Mycology laboratory of dermatology department, Ain Shams University) and Tarek Mostafa Abd-Al Azez (M.cs. Microbiology, Al-Azhar University, National Cancer Institute, Microbiology department) for their help in this study.

References:

- 1. **Aba AlKhail A. A. 2005.** Antifungal activity of some extracts against some plant pathogenic Fungi. Pak. J. Biol. Sci. 8: 413-417.
- Abd-Alla M. S., Atalia K. M. and El-Sawi M. A. M. 2001. Effect of some plant waste extracts on growth and aflatoxin production by *Aspergillus flavus*. Annals Agri. Sci. 46: 579-592.
- 3. **Abdel-Kader, H.A. and Seddkey,S.R. 1995.** *in vitro* study of the effect of some medical plants on the growth of some dermatophytes. Assiut Veterinary Medical Journal 34:67.
- 4. **Abbas-Nasar S.M., Halkman A.K. 2004.** Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. International Journal of Food Microbiology, 97: 63-69.

- Adegoke, S. A., Opata, O. M. and Olajide, J. E. 2010. Antimicrobial activity of the aqueous, methanol and chloroform leaf extracts of *Cissus multistriata*. African Journal of Biotechnology Vol. 9 (8), pp. 1168-1172. Available online at http://www.academicjournals.org/AJB.
- Aiyelagabe O.O. 2001. Antibacterial activity of *Jatropa multifida* roots. Fitoterapia, 72: 544-546.
- 7. **Ali-Shtayeh M. S. and Abu Ghdeib I. Suheil. 2002.** Antifungal activity of plant extracts against dermatophytes. <u>Mycoses</u>, <u>Volume 42 Issue 11-12</u>, Pages 665 672.
- 8. **Amer S., Aly M.M., Sabbagh S. 2006**. Biocontrol of dermatophytes using some plant extracts and actinomycetes filtrates. *Egyptian J. Biotechnol.*, 330-315.
- Amiot M.J. and Nguyen C. 2000. Antimicrobial effect of Rosemary extract. J. Food Protect. 63: 1359-1368.
- 10. **Anandaraj, M. and Leela, N.K. 1996.** Toxic effect of some plant extracts on *Phytophthora capsici*, the foot root pathogen of black pepper. Indian Pathology 49(2): 181-184.
- 11. **Atlas, R.M. 1993.** Handbook of microbiology media. pp.278,538,785. In Laurence,C. and Parks, L.c. eds. Paks, CRC press.
- 12. **Caceres A., Jauregui E., Herrera D. and Logemann H. 1991**. Plants used in Guatemala for the treatment of dermatomucosal infections: Screening of 38 plants extracts for anticandidal activity. J. Ethanopharmacol. 33: 277-283.
- 13. Clark A. M. and Hufford C. D. 1992. Discovery and development of novel prototype antibiotics for opportunistic infections related to acquired immunodeficiency syndrome. In: Human Medicinal Agents from Plants. Kinghorn, A., and Balandrin, M., (Ed.) American Chemical Symposium Series 534, American Chemical Society, New York, USA. pp. 229-241.
- 14. **Cowan M. M. 1999.** Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-582.
- 15. Dash B.K., Faruquee H.M., Biswas S.K., Alam M.K., Sisir S.M. and Prodhan U.K. 2011. Antibacterial and Antifungal Activities of Several Extracts of *Centella asiatica* L. against Some Human Pathogenic Microbes Life Sciences and Medicine Research, Volume: LSMR-35.
- 16. De Hoog G.S., Guarro J. Gene and Figueras M.J., 2000. Atlas of clinical fungi 2nd edition.
- Del Campo J., Amiot M.J. and Nguyen C. 2000. Antimicrobial effect of Rosemary extract. J. Food Protect. 63: 1359-1368.
- 18. **Dewitte-Orr S.J., Zorzitto J.R., Sutton L.P. and Bols N.C. (2005).** Cycloheximide. Fish. Shell. Immunol. 18, 279.
- 19. El-Assiuty E. M., Bekheet F. M., Fahmy Z. M., Ismael A. M. and El-Alfy T. S. M. 2006. Potentiality of some isolated compounds from Halfa Barr (*Cymbopogon proximus*

- Stapf.) against the toxigenic fungi *Fusarium verticillioides* and *Aspergillus flavus*. Egypt. J. Phytopathol. 34: 75-84.
- 20. Gayathri Gunalan , Saraswathy A. and Vijayalakshmi Krishnamurthy.2011. Antimicrobial Activity Of Medicinal Plant Bauhinia variegata Linn. International Journal of Pharmacy and Biological Sciences, IJPBS, Volume 1, Issue 4, 400-408.
- 21. **Grane M. and Ahmed S. 1988.** Handbook of Plants with Pest Control Properties, John Wiley and Sons, New York, USA. p. 431.
- 22. **Ibrahim M. B. 1997.** Anti-microbial effects of extract leaf, stem and root bark of *Anogeissus leiocarpus* on *Staphylococcus aureaus*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus vulgaris*. *J. Pharma*. *Devpt.*, vol. 2, pp. 20-30.
- 23. **Kazemi M., Rostami H. and Shafiei S. 2012.** Antibacterial and Antifungal Activity of some Medicinal Plants from Iran. Science Alert. April 14, 2012.
- 24. **Kurita N., Makoto M., Kurane R. and Takahara Y. 1981.** Antifungal activity of components of essential oils. Agric. Biol. Chem. 45: 945-952.
- 25. **Lahlou M. 2004.** Methods to study the Phytochemistry and bioactivity of essential oils. *Phytotherapy Research* 18, 435–448.
- 26. Loiy Elsir, Ahmed Hassan, Hasnah Mohd Sirat, Sakina M. Ahemd Yagi, Waleed S. Koko and Siddig Ibrahim Abdelwahab. 2011. *In vitro* Antimicrobial activities of chloroformic, hexane and ethanolic extracts of *Citrullus lanatus* var. *citroides* (Wild melon). Journal of Medicinal Plants Research Vol. 5(8), pp. 1338-1344.
- 27. **Mahesh B. and Satish S. 2008.** Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agri. Sci. 4 (S), 839-843.
- 28. **Mann A., Banso A. and Clifford L.C. 2008.** An antifungal property of crude plant extracts from Anogeissus leiocarpus and Terminalia avicennioides. Tanzania J. Health Res. 10 (1) 34-38.
- Meena A. K., Kaur R., Singh B., Yadav A. K., Singh U., Sachan A., Pal B. and Rao M. M. 2009. Review on antifungal activities of Ayurvedic medicinal plants. Drug Invention Today. 2: 146-148.
- 30. **Mueller M.S. and Mechler E. 2005.** Medicinal Plants in Tropical Countries: Traditional Use Experience Facts. , Stutgart, Germany.
- 31. Neena Jain and Bettina C. Fries. 2011. Fungal Infections in Humans. Published Online: 15 SEP, DOI: 10.1002/9780470015902.
- 32. **Ogundipe O., Akinbiyi O. and Moody J.O. 1998.** Antibacterial activities of essential ornamental plants. Nigeria J. Natural Products & Medicine 2, 46-47.
- 33. **Owolabi, O.J., Omogbai, E.K.I. and Obasuyi, O. 2007**. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. <u>African Journal of Biotechnology</u>. <u>Vol 6,</u> 1684-5315.
- 34. **Pavel M. and Alecu F. 2008.** Antifungal activity of *Thymus serpyllum* essential oil against *Candida albicans* and Candida non-albicans clinical isolates.18th European

- Congress of Clinical Microbiology and Infectious Diseases (ECCMID)19.04.2008 22.04.
- 35. **Pirzada A.J., Shaikh W., Usmanghani K. and Ejaz Mohiuddin. 2010.** Antifungal activity of *Dodonaea viscose* extract on pathogenic fungi isolated from superficial skin infection. Pak. J. Pharm. Sci., Vol.23, No.3, July, pp.337-340.
- 36. **Pitt. I. John 1991.**A laboratory guide to common *Peniciliium* species.Book.
- 37. **Pretorius J., Zietsman P. and Eksteen D. 2002.** Fungi toxic properties of selected South Africa plant species against plant pathogens of economic importance in agriculture. Annals Appl. Biol. 141: 117-124.
- 38. Sawsan Abd-Ellatif, Salwa M. Abdel Rahman and Sahar F. Deraz. 2011. Pomosing antifungal effect of some folkoric medical plants collected from El- Hammam habitat, EGYPT against dangerous pathogenic and toxinogenic fungi.Vol. 6, No. 9, September, ISSN 1990-6145 ARPN Journal of Agricultural and Biological Science.
- 39. Sharma R. A., Chandrawat P., Sharma S., SHARMA D., Sharma B. and Singh D. 2010. Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India.5 (3): 441 444.
- 40. Shittul L.A.J., Bankole M.A., Ahmed T., Bankole M.N., Shittul R.K., Saalu C.L., Ashiru. 2007. Antibacterial and Antifungal Activities of Essential Oils of Crude Extracts of *Sesame Radiatum* against Some Common Pathogenic Micro-Organisms Biol. Sci., 13: 1023-1029.
- 41. **Silva, N.C.C. and Fernandes-Junior, A. 2010**. Biological properties of medicinal plants: a review of their antimicrobial activity. Journal of venomous animals and toxins including tropical diseases 16, 402-413.
- 42. Takamasa Kaneko, Koichi Makimura, Michiko Abe, Ryoko Shiota, Yuka Nakamura, Rui Kano, Atsuhiko Hasegawa, Takashi Sugita, Shuichi Shibuya, Shinichi Watanabe, Hideyo Yamaguchi, Shigeru Abe, and Noboru Okamura, 2007. Revised Culture-Based System for Identification of *Malassezia* Species. J, Clin Microbiol. November; 45(11): 3737–3742.
- 43. **Taplin D. 1965.** The use of gentamicin in mycology. J. Invest. Dermat., 45; 549-550.
- 44. **Valeria F.M., Preve L., Tullio V. 1996.** Fungi responsible for skin mycoses in Turin (Italy). *Mycoses*, **39**: 141-150. Wikipedia, the free encyclopedia. 2010. **Mycosis.**
- 45. **Wilson C. L., Solar J. M., Ghaouth A. E. l. and Wisniewski M. E. 1997.** Rapid evaluation of plant extracts and essentials oils for antifungal activity against Botrytis cinerea. Plant Dis. 81: 204-210.
- 46. **Woldemichael G.M., Wachter G., Singh M.P. 2003.** Antibacterial diterpenes from *Calceolaria pinifolia*. Journal of Natural Products, 66: 242-246.
- 47. **Zaker, M. and Mosallanejad, H. 2010**. Antifungal activity of some plant extracts on *Alternaria alternata*, the causal agent of alternaria leaf spot of potato. <u>Pak J Biol Sci.</u> 21:1023-9.

" مراسة استطلاعية لبعض المستخلصات النباتية ضد بعض مسببات الأمراض الفطرية للإنسان."

أحمد محمد السيد رجب 1 ، حسين حسنى الشيخ 2 ، إيمان محمد أحمد الطاهر 3 بسمة حمدى أمين

أ قسم النبات والميكروبيولوجى – كلية العلوم – فرع البنات – جامعة الأرهر وسم النبات والميكروبيولوجى – كلية العلوم – فرع البنين – جامعة الأرهر 2 قسم النبات والمركز الإقليمى للفطريات وتطبيقاتها – جامعة الأرهر 3

الملخص العربي

يلعب المضاد الفطرى دوراً هاماً فى علاج الأمراض الخطيرة الـتى تصيب الإنسان ولـذلك فـإن الحصـول على المضاد الفطـرى من المصـادر الطبيعية يعد أفضل من المركبـات التخليقيـة. لـذلك تم إجـراء تلك الدراسة للحصـول على نـواتج أيضـية ثانوية فعالة ضد الأمـراض الفطرية من بعض المستخلصات النباتية.

تم الحصول على 32 مستخلص نباتى من 16 نبات صحراوى، 16 نبات صحراوى، 16 نبات منزرعة باستخدام ميثانول ، ميثانول : ماء (1:1)، ماء ، كلوروفورم، بينزين. كما تم إختبار تلك المستخلصات ضد 46 عزلة فطرية ممرضة للإنسان تتمثل فى 12 عزلة خميرة ،2 عزلة فطريات شبيهة الخميرة و 32 عزلة فطريات المستخدمة وجد أن كلورفورم هو الأكثر فعالية فى استخلاص المواد الفعالة يليه بنزين ثم ميثانول: ماء (1:1) يليه ميثانول ، بينما المستخلصات المائية كانت الأقل نشاطاً فى عملية الاستخلاص.

أظهـرت مستخلصـات كلوروفـورم أعلى نشـاط تثـبيطى ضد النمو الفطرى للعزلات محل البحث. وقد وجد أن الزعتر البرى (نبات صـحرواى) وشبت (نبات منزرع) أظهرا نشاطاً واضحاً ضد كل من فطرة *أسـبرجيللس* تامريـاى 1 المعزولة من العيـون وفطـرة *بنسـيليوم مارنيفـاى* المعزولة من الدم.

وتلك الدراسة توضح استخدام المستخلصـات النباتية كمضـادت فطرية فعالة.