SURVEY OF SOME PLANT EXTRACTS AGAINST CERTAIN HUMAN FUNGAL PATHOGENS
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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).
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 against 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), Ocimum basilicum (basil), Lippia sidoides (rosemary-pepper), Plectranthus amboinicus (mint), Eucalyptus 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 (±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 Penicillium species were identified by using A laboratory guide to common Penicillium 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 μm.

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, Cymbopogon 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 piperita, 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 μl 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.

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Causative fungus</th>
<th>Isolation sites</th>
<th>Disease</th>
<th>Gender</th>
<th>Age</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida albicans 1</td>
<td>Sputum</td>
<td>Chest infection</td>
<td>Female</td>
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<td>Otomycosis</td>
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<td>Urine</td>
<td>Urinary tract infection</td>
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<td>Vulvovaginitis</td>
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<td>Vagina</td>
<td>Vulvovaginitis</td>
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<td>Vagina</td>
<td>Vulvovaginitis</td>
<td>Female</td>
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<td>45</td>
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<tr>
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<tr>
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<td>Male</td>
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<tr>
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<td>Onychomycosis</td>
<td>Female</td>
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<td>Eye</td>
<td>Keratitis</td>
<td>Male</td>
<td>53</td>
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<tr>
<td>28</td>
<td>Aspergillus tamarii 1</td>
<td>Eye</td>
<td>Keratitis</td>
<td>Male</td>
<td>55</td>
</tr>
<tr>
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<td>Blood</td>
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</tr>
<tr>
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<td>Onychomycosis</td>
<td>Female</td>
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<td>Blood</td>
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<td>35</td>
</tr>
<tr>
<td>38</td>
<td>Microsporum canis 1</td>
<td>Hair scalp</td>
<td>Tinea capitis</td>
<td>Boy</td>
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Fig. (1): Light microscopy micrograph of yeasts; *Candida albicans* (A), *Candida famata* (B), *Candida kreusi* (C), *Candida tropicalis* (D), *Cryptococcus laurentii* (E), *Malassezia sympodialis* (F) and *Rhodotorula glutinis* (G). Scale bar =10 μm.
SURVEY OF SOME PLANT EXTRACTS AGAINST CERTAIN ...
Fig. (3): Light microscopy micrograph of Pencillia; *Penicillium duclanxii* (A), *Penicillium aurantiogriseum* (B), *Penicillium chrysogenum* (C), *Penicillium fellutanum* (D), *Penicillium janczewskii* (E), *Penicillium marneffei* (F). Scale bar =10 μm.
Fig. (4): Light microscopy micrograph of *Microsporum canis* (A), *Trichophyton mentagrophytes* (B), *Trichophyton rubrum* (C), *Mucor hemalis* (D), *Abisidia corymbifera* (E), *Geotrichum candidum* (F), *Syncephalastrum racemosum* (G) and *Fusarium solani* (H). Scale bar =10 $\mu$m.
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 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 laurentii* (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* exhibited good results against *Aspergillus terreus* 1 (4.5 cm) and *Mucor hemalis* (4.5 cm), while, *Solenostemma 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 niger* and *Candida albicans*) were tested. The chloroform extract of the fruits exhibited the highest antibacterial activity, also, *A. niger* 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 pathogens (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.

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**References:**


Stapf.) against the toxigenic fungi *Fusarium verticillioides* and *Aspergillus flavus*. Egypt. J. Phytopathol. 34: 75-84.


دراسة استطلاعية لبعض المستخلصات النباتية ضد بعض مسببات الأمراض الفطرية للإنسان.

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الملخص العربي

يلعب المضاد الفطري دوراً هاماً في علاج الأمراض الخطيرة التي تصيب الإنسان ولذلك فإن الحصول على المضاد الفطري من المصادر الطبيعية يعد أفضل من المركبات التخليقية. لذلك تم إجراء تلك الدراسة للحصول على نوائح أفضلي ثانوية فعالة ضد الأمراض الفطرية من بعض المستخلصات النباتية.

تم الحصول على 32 مستخلاص نباتي من 16 نبات صحراوي، 16 نباتات منزوعة باستخدام ميثانول، ميثانول: ماء (1:1)، ماء، كلوروفورم، بنتزين. كما تم اختيار تلك المستخلصات ضد 46 عزلة فطرية مرضية للإنسان تتمثل في 12 عزلة خميرة، 2 عزلة فطريات شبيهة الخميرة، و32 عزلة فطريات خيطية. ومن بين الخمس مذيبات المستخدمة وجد أن كلوروفورم هو الأكثر فعالية في استخلاص المواد الفعالة ليليه بنتزين ثم ميثانول: ماء (1:1) يليه ميثانول، بينما المستخلصات المائية كانت الأقل نشاطاً في عملية الاستخلاص.

أظهرت مستخلصات كلوروفورم أعلى نشاط تثبيطي ضد النمو الفطري للعزلات محل البحث. وقد وجد أن الزعتر البري (نبات صحراوي) وشيت (نبات منزوع) أظهرا نشاطاً واضحاً ضد كل من فطرة أسبيرجيلس
تامرياي 1 المعزولة من العيون وفطرة نسيليم مارنوفاي المعزولة من الدم.

وتلك الدراسة توضح استخدام المستخلصات النباتية كمضادات فطرية فعالة.
SURVEY OF SOME PLANT EXTRACTS AGAINST CERTAIN