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Screening of Some Plant Extracts against Alternaria sp. Isolated from Foot Infections in Cancer Patients

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Abstract: Microorganisms are ubiquitous in nature. A large number of microbes are present in our environment. The human body occurs in dynamic equilibrium with these microbes. Infection occurs when a microbe penetrate the body surface of tissues. In these it multiplies and the cumulative effects infections damage of disrupt tissues and organs and a disease result.

Alternaria sp, Aspergillus Niger, A. flavus, A. fumigatus and Curvularia sp. were the most prevalent fungi causing nail infection in human beings causing onychomychosis.

In vitro evalution was conducted for sensitivity testing with 8 different plant extract for the inhibition of hyphal growth and spore formation in Alternaria sp.

Evolution of antifungal activity was carried out by disc diffusion method. Mycosis is common in cancer patients with the emergence should be more aware and physicians should be of new effective systemic and tropical antifungal therapy, study of the epidemiology and extent of impact of this infection is important. In general, people should be more aware and physicians should be more clinically alert and should target their clinical suspicion to the higher risk group to initiate prompt investigation and treatment.

Key words: Antifungal activity, in vitro evalution, fungal infection, plant extract.

Introduction

Infection of nail caused by fungi or ungula mycoses are generally called "Onychomycosis". Onycomycosis can be divided into four clinical presentations: distal subungal (the most common form of the disease), proximal subungal (the most comman form found in patients with human) and superficial and total dystrophic onycomycosis [1-2]. Foot fungus will begin to develop between the toes before spreading to the arch, sole, heel and the rest of foot. It will sometimes cause the bottom of the feet to turn red, scaly and flaky [3]. Immunocompromised individuals are more prone to infections. Some types of cancers and treatments can weaken our immune system. They stop our bone marrow making blood cells that help fight infection. This increases risk of getting an infection [4].

Fungi are increasingly recognized as major pathogens in critically ill patients Candida sp and Cryptococcus sp are the yeasts most frequently isolated in clinical practice. The most frequent filamentous fungi (moulds) isolated are Aspergillus sp, but Fusarium sp, Scedosporium sp, Penicillium sp and Zygomycetes are increasingly seen [5]. Aspergillus's sp is the most commonly isolated from fungal infection.

Plants contain various active molecules like-Alkaloids, Terpenoids, Steroids, Fatty acids, Flavinoids. Flavinoid are secondary metabolite commonly known for their antioxidant activity. They are also referred to as bioflavonoid. Flavinoid have both antimicrobial and anticancer properties.

Alkaloids also have antioxidant activity. They also provide the health benefits against cancer [6]. So Flavinoids and Alkaloids containing plants can be used in the treatment of mycosis in cancer patients, so in the

present study alkaloid as well as flavanoid containing plants are selected for determining their antimycotic potential. The Present study is designed to evaluate the antimycotic activity of the flavinoid and alkaloid containing plant extracts and the possibilities to use different plant extracts as a preventive means against fungi causing mycosis in cancer patient. So as to find agent which is less harmful than synthetic chemicals and wouldn't cause any side effect in patients receiving chemotherapy and other medication.

The treatment of human mycosis has been great challenge before the clinician and dermatologist. In at one hand the opportunistic fungal infection are increasing with alarming rate, while on other hand; allergic reaction of the skin increasing day to day. But these potent antimycotics are in the hands of rich and not responding to the new spectrum of opportunistic fungal infections which are common in immunocompromised hosts. Therefore, to combat newly borne spectrum of fungal infections, step should be taken to make begins of successful pharmaceutical research available to all and especially to those who are in the greatest need [4].

In recent years, there has been an increasing search for new antifungal compounds due to the lack of efficacy, side effects and or resistance associated with some of the existing drugs [7-8] .Much attention paid to plant derived antifungal compounds [9] .Based on the knowledge that plants have their own defense system against fungal pathogens [10]. Natural products obtained from many plants have been scientific interest [11]. The Present study is designed to evaluate the antimycotic activity of the flavinoid and alkaloid containing plant extracts and the possibilities to use different plant extracts as a preventive means against fungi causing mycosis in cancer patients.

Materials and Methods

Materials:

Preparation of swabs:

Swabs with a cotton wool tipped applicator were prepared and put inside culture tubes and sterilized by hot air oven for 1 hrs. At 140^0C temperature. For fungal infection the sample is inoculated on sabauraud's dextrose agar plates to which streptomycin was added to inhibit bacterial growth. The plates were incubated for 48-72 hrs at 37^0c after which the colonies were studied.

Test organisms:

Fungi strains isolated from patients i e Alternaria sp, was used & maintained on Sabrauds dextrose agar in Department of Microbiology, CHRI, Gwalior.

Methods

Collection of samples

In the present study cases were studied. Patient with symptoms suggestive of mycosis early in the cancer patients were examined by doctors in the dermatologist department. The samples were collected from the patients by a sterile scalpel blade. To minimize air borne contamination sample were immediately placed in sterilized plastic bags for storage and transport to the laboratory.

Table. 1 Fungal species described as causes of mycosis

Dermatophyte fungi	Yeast	Nondermatophyte fungi
Epidermatophyton floccosum	*Candida abdicans*	*Acermonium sp*
Trichophyton concentric	*Candida famata*	*Aspergillus sp*
Trichophyton mentagrophyte	*Candida guilliermondii*	*Alternaria sp*
Trichhophyton minima	*Candida parapsilosis*	*Helminthosporium sp*
Trichophyton rubrum	*Candida tropicalis*	*Fusarium sp*
Trichophyton shoenleinii	*Candida sake*	*Curvularia sp*
Trichiphyton soudanense		*Cryptococcus sp*
Trichophyton tonsurans		*Scedosporium sp*
Trichophyton violence		

Plant collection & Preparation of extracts

The fresh leaves, of white vinica rosea, pink vinica rosea, jasminum sambac, jasminum grandiflorum, hibiscus rosa, calendula officinalis , citrus laminai plant were collected from Gwalior District in Madhya Pradesh, India .The plant parts were thoroughly washed with tap water followed by distill water and were shade dried. The dried leaves were crushed with a mortar and pestle and fine powder was prepared. The powdered leaves were weighed and twenty five gram of powdered leaves was extracted with the help of Soxhlet apparatus. For soxhlet extraction, thimble was prepared .Powdered leaves (25 g) are placed in an extraction thimble which is then placed in a Soxhlet extraction apparatus. Methanol (250ml) is added to the thimble allow to stand overnight. Next morning the solvent is refluxed through the extraction thimble for 12 hours. The methanol solution is evaporated, and the dark gummy residue obtained is dried in Calcium Carbonate chamber. The dark green solid obtained is crushed to provide a methanol extract powder. The dried extract was than stored in airtight jars at 4^0C for further assays.

Preparation of sample:

The 500 mg of plant Extracts were dissolved in 1000 µl of methanol .This solution was vortexed over vortex mixture to get homogenize preparation.40 µl of above described plant extract was added to each well of plate .These plates were kept overnight for evaporation of methanol and diffusion of extracts . This procedure was also helpful in checking contamination.

Inoculum preparation:

The moulds inoculum preparation of conidial or sporangiospores suspensions must be adjusted using a spectrophotometer with a test inoculum in the range $0.4x10^4$ to $5x10^4$ CFU/ml. The optical density (OD) at 530nm required is dependant on the conidial or sporangiospores size of the mould being tested; i.e. for Aspergillus sp. the OD = 0.09 - 0.11; for Alternaria sp. the OD = 0.2. 0.05%Tween 80 was added as wetting agent to facilitate the preparation of inoculum.

Antifungal activity:

The media was taken in petridish ,the cork borer was used for creation of well in the solidified media .Cork borer was sterilized with the help of alcohol and incineration after every single use .A total of four wells were punched in the petridish having 90 mm diameter. Antifungal activity of the extracts was tested using the agar well diffusion method. Twenty ml Mueller-Hinton agar of was taken into sterilized petridish and allowed to solidify. Afterward 7 mm wells were punched on plate with cork borer, four wells were punched in each plate. 40µl of plant extracts which was reconstituted in methanol was transferred to each well. For the proper diffusion of extracts and evaporation of methanol, plates were kept for 24 hrs and used next day for antifungal activity. This process also checks the possible contamination during plating and loading of extracts. The MHA was than seeded with test fungal strain by using sterile cotton swabs. A sterile cotton swab was dipped into the suspension .Pressed firmly against the inside wall of the tube just above the fluid level and rotated to remove excess liquid. The swabs were streaked over the entire surface of the medium three times, rotating the plates approximately 60 degree after each application to ensure an even distribution of the inoculum. Finally swab was streaked all around the edge of the agar surface. All the dishes were than incubated at 28 ± 2^0C for 48 hrs. A set of control was also run in same way for each test organism using Amphotericin B.

Recording & interpreting of results

Diameter of zone of inhibition against test fungi was measured (in mm) with antibiotic zone measuring scale (Hi Media) on the under surface of the plate without opening lid & an average of three independent determination was recorded.

Results

In the present study, a total of 60 samples from cancer patients suffering from onychomycosis were collected, a positive diagnosis of foot fungal infection was obtained in 48 cases and 12 cases were negative. The group consisted of 12 (20%) female and 48 (80%) male. The age at diagnosis ranged from 20 years to 70 years. Present investigation shows that Alternaria sp., isolated from the samples.

The antifungal activity pattern of different plants leaf as Citrus laminai, Calendula officinalis, Hibiscus rosa, Jasminum grandiflorum, Jasmium sambac, Pink Vinca rosea(Cantharanthus roseus var.rosear), White Vinca rosea (Cantharanthus roseus var.albus) showed activity with varying magnitude. The zone of inhibition ≥10mm diameter was taken as positive result. The results of antimicrobial susceptibility pattern obtained are presented in Table 3.

The diameter of inhibition zone for different fungi was varied with different plant extracts. Out of all the plant extracts, Jasminum grandiflorum has shown maximum antifungal activity. All the tested fungi exhibited resistance against Pink vinica rosea (Cantharanthus roseus var.rosear), and White vinica rosea (Cantharanthus roseus var.albus) leaf, Calendula officinalis leaf.

The sensitivity test shows that Amphotericin B is mildly effective against various fungus cultures. Infectious diseases, including mycosis, due to the increasing development of antimicrobial resistance as well as the appearance of undesirable effect of some anti-fungal agent.

In the present study Jasminum grandiflorum, Jasminum sambac, Hibiscus Rosa, Citrus leminai, showed antimicrobial activity against test microorganisms (Table 3). Among tested plant species we found that the methanolic extracts of Jasminum grandiflorum showed highly against, Alternaria sp, Jasminum sambac is also highly effective against Alternaria sp. Hibiscus rosa and Citrus laminai are effective against Alternaria sp. Antimicrobial property of Pink Vinca rosea (Cantharanthus roseus var.roseus) White Vinca rosea (Cantharanthus roseus var.albus) Calendula officinalis can be used as effective measure to control the fungal pathogen.

Table-3 Antifungal activity of plant extracts

S. No.	Name Of plant extracts	Zone of inhibition Alternaria sp.
1.	Pink Vinca rosea leaf	R
2.	WhiteVinca rosea leaf	R
3.	Citrus laminai leaf	18.5
4.	Hibiscuss rosa leaf	19
5.	Calendula officinalis leaf	R
6.	Jasminum sambac leaf	40
7.	Jasminum grandiflorum leaf	43
8.	Amphotericin B	30

R= resistant

Discussion

Vinica rosea is known to have antiallergic, anti-inflammatory and anticancer activity. It has more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat different types of cancers. It also have alkaloids and which have antimycotic properties so in the present study extract of this plant is examined against fungi isolated in the cancer patients. So as to develop the formulation, which can be used in the treatment of mycosis in cancer patient.

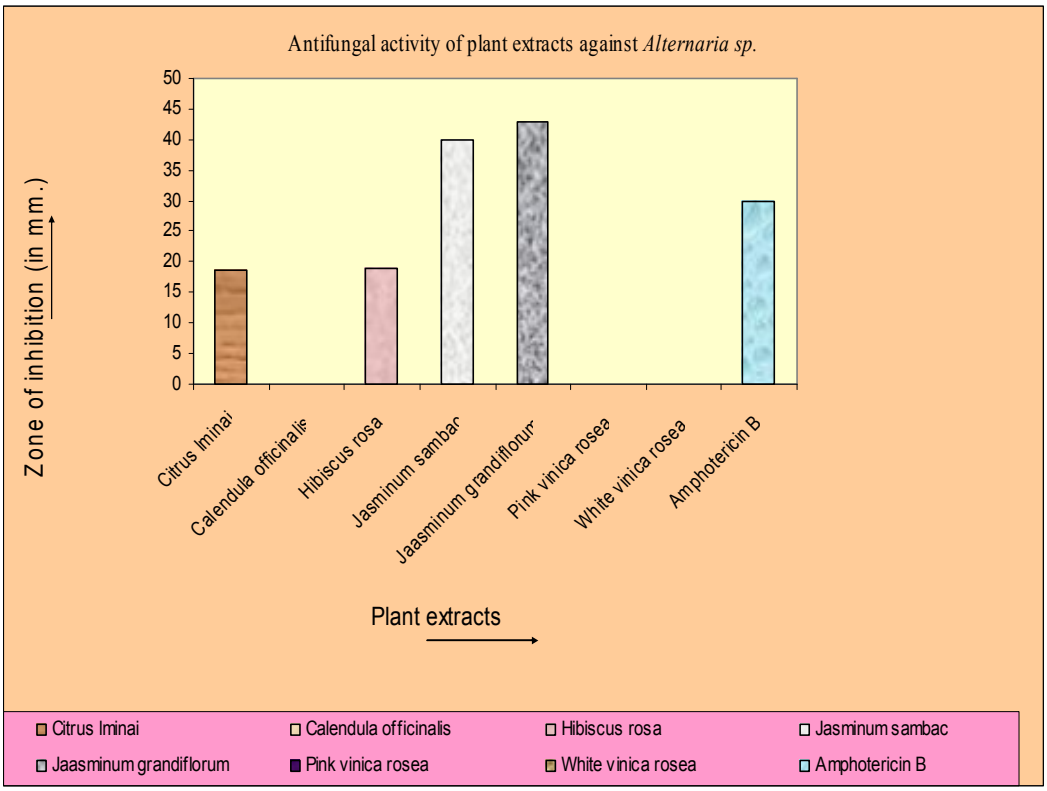
All parts of Hibiscus plants are used traditionally,due to their soothing (demulcent) and astringent properties, the flowers and leaves have been traditionally used to treat conditions such as cancer and topically to treat skin infections .

Citrus laminai fruits contain various kinds of flavinoids such as flavanone glycoside, flavone glycoside, and polymethoxyflavone. The flavonoids in lemon fruits (Citrus laminai) have been reported to be such flavanone glycosides as eriocitrin (eriodictyol 7-O-β-rutinoside) and hesperidin (hesperetin 7-O-β-rutinoside), naringin (naringenine-7-rhamnosidoglucoside), and such flavanone glycosides as diosmin (diosmetin 7-O-β-rutinoside) and 6,8 C-diglucosyldiosmetin (Miyake,1997; Kawai,1999), all of which are supposed to have a number of positive health effects in the prevention of lifestyle-related diseases, and to have antiinflammatory, anti-cancer, and antiviral activities based on their antioxidant activity anti-infectious, antibacterial (spores), antiseptic disinfectant. Essential oil of Calendula officinalis, reported to have a 100% inhibitory effect against fungi[12]. But in the present study, calendula officinalis was not found effective against tested fungus.

The Jasminum grandiflorum leaves are largely used in folk medicine to prevent and treat cancer .It is widely used in aurveda as a skin diseases and wound healing. It has anti-inflanmatory, antimicrobial agent, antitumor, and anti-carcinogenic properties. In jasminum grandiflorum triterpenes, flavinoid, alkaloid, tannins, saponins are present. The flavinoid and triterpenoids are known to promote the wound healing mainly due to their antimicrobial properties. In the present study jasminum grandiflorum showed antimycotic effect also and significantly retarted the growth of fungi Alternaria sp.

Jasmine (Jasminum sambac, sans- mallika) is extensively used in manufacturing high grade aromatherapy. Juices from the leaves of J. sambac are applied to treat ulcers, remove corns, it show anti-inflamation and anti-cancer and also show anti-oxidant property [13-14]. Jasminum sambac, Jasminum grandiflorum, Hibiscus Rosa, Citrus leimiai are show activity against test-organisms.

Based on the above finding we can say that the plant contain various active molecules. Amongst tested plant jasminum grandiflorum showed significance activity. It is known to have anticancerous properties and also found effective against fungi causing onychomycosis in cancer patient thus this can be used in the treatment against fungi causing mycosis in cancer patients without any significant side effect. Though further studies are needed, thus may help to develop effective formulation but this is a pulmonary study further study and in-vitro trials are needed.



Conclusion

The human body occurs in dynamic equilibrium with these microbes. Infection occurs when a microbe penetrate the body surface of tissues. In these it multiplies and the cumulative effects infections damage of disrupt tissues and organs and a disease result. In vitro evalution was conducted for sensitivity testing with 8 different plant extract for the inhibition of hyphal growth and spore formation in Alternaria sp. Evolution of antifungal activity was carried out by disc diffusion method. Mycosis is common in cancer patients with the emergence should be more aware and physicians should be of new effective systemic and tropical antifungal therapy, study of the epidemiology and extent of impact of this infection is important. In general, people should be more aware and physicians should be more clinically alert and should target their clinical suspicion to the higher risk group to initiate prompt investigation and treatment.

Emergence of dreaded diseases like AIDS and CANCER are responsible for increase in number of secondary infections generally caused by opportunistic fungi due to their immunocompromising capacity. The azoles and other antifungal drugs often fail to respond well to these infections. Therefore, there has been greater need to search for alternative antifungal agent from microbes or plants.

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