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1. Iqbal Ahmad, Maryam Zahin, Farrukh Aqil, Sameena Hasan, M Sajjad A Khan and Mohd Owais (2008). Bioactive compounds from *Punica granatum, Curcuma longa* and *Zingiber officinale* and their therapeutic potential. *Drugs of the Future*. 33(4):329-346 (Publisher-Prous Science, Spain; ISSN: 0377-8282 (print version) 2013-0368 (electronic version); impact factor 0.5).
Papers presented in the proceedings/conferences:

1. Presented paper entitled “Biofilms of Candida albicans, prevented and destroyed by plant essential oils: A new hope for combating drug-resistant Candida” by Mohd Sajjad Ahmad Khan and Iqbal Ahmad. The paper was nominated for the Young Scientist Award (for Mohd Sajjad Ahmad Khan) under section of Medical Sciences (including physiology), in the proceedings (on 14.10.2011, p-43) of the 99th session of The Indian Science Congress Association at KIIT University, Bhuwaneswar, Orissa, India.

Research papers communicated:

1. Mohd Sajjad Ahmad Khan and Iqbal Ahmad (2011) In vitro inhibition of growth and virulence factors in pathogenic yeasts by certain essential oils and active compounds. Indian journal of Medical Research. (Publisher-Indian Council of Medical research; impact factor 1.826), under review.


3. Mohd Sajjad Ahmad Khan and Iqbal Ahmad (2011) In vitro influence of essential oils on germ tube formation, cell surface hydrophobicity, production of proteinase and haemolysin in Candida albicans. Pharmaceutical Biology (Publisher-Informa Healthcare; impact factor 0.8).
Antibiofilm activity of certain phytocompounds and their synergy with fluconazole against Candida albicans biofilms

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Objectives: The aim of this study was to evaluate four phytocompounds (cinnamaldehyde, citral, eugenol and geraniol) for their in vitro inhibitory activity against pre-formed biofilms of Candida albicans alone or in combination with fluconazole and amphotericin B. These compounds were also tested at subinhibitory concentrations for their ability to inhibit biofilm formation.

Methods: The XTT reduction assay, light microscopy and scanning electron microscopy (SEM) were employed to determine the inhibitory effect of the test compounds on biofilms. A chequerboard method was used for combination studies.

Results: Both clinical and reference strains of C. albicans (C. albicans 04 and C. albicans SC5314, respectively) displayed formation of strong biofilms. Pre-formed Candida biofilms showed >1024x increased resistance to antifungal drugs and 2x increased resistance to cinnamaldehyde and geraniol, but no increased tolerance of eugenol. The test compounds were more active against pre-formed biofilms than amphotericin B and fluconazole. At 0.5x MIC, eugenol and cinnamaldehyde were the most inhibitory compounds against biofilm formation. Light and electron microscopic studies revealed the deformity of three-dimensional structures of biofilms formed in the presence of sub-MICs of eugenol and cinnamaldehyde. The cell membrane appeared to be the target site of compounds in both planktonic and sessile C. albicans cells, as observed by SEM. Combination studies showed that synergy was highest between eugenol and fluconazole (fractional inhibitory concentration index=0.14) against pre-formed biofilms of C. albicans SC5314.

Conclusions: Promising antibiofilm activity was displayed by eugenol and cinnamaldehyde, which also showed synergy with fluconazole in vitro. Further evaluation in in vivo systems is required to determine whether these findings can be exploited in treating biofilm-associated candidiasis.

Keywords: eugenol, cinnamaldehyde, scanning electron microscopy, XTT reduction assay

Introduction

The majority of manifestations of candidiasis are associated in one way or another with the formation of Candida biofilms on the surfaces of inert or biological surfaces. Sessile (biofilm) cells display unique phenotypic traits in comparison with planktonic cells. The most notable of these is that sessile cells are notoriously resistant to antimicrobial agents and withstand host immune defences, and this is the main reason why biofilm-associated infections are frequently refractory to conventional antibiotic therapy. The decreased susceptibility of sessile cells to antimicrobial agents, including amphotericin B, fluconazole, itraconazole and ketoconazole, compared with that of planktonic cells has been reported extensively over the past decade. To overcome the problem of host toxicity and drug resistance associated with monotherapy, two-drug combination strategies have been attempted both for planktonic and biofilm cells of C. albicans, but disparate effects have been observed. Given these concerns, identifying antifungal agents that are effective against Candida biofilms alone or in synergy with fluconazole or amphotericin B is of great importance. Plants are known to produce phytochemicals that attenuate biofilm development through specific mechanisms. Therefore, in this study, we evaluated four phytocompounds of different chemical nature [phenyl aldehyde (cinnamaldehyde; 3-phenylprop-2-enal), phenyl propanoid (eugenol; 4-allyl-2-methoxyphenol) and terpenoid (citral; 3,7-dimethylocta-2,6-dien-1-ol) and geraniol; 3,7-dimethylocta-2,6-dien-1-ol)] for their ability to eradicate established biofilms and to inhibit biofilm formation at sub-MICs in strains of C. albicans. The antifungal drugs...
In vitro antifungal, anti-elastase and anti-keratinase activity of essential oils of Cinnamomum- , Syzygium- and Cymbopogon-species against Aspergillus fumigatus and Trichophyton rubrum

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ABSTRACT

This study was aimed to evaluate effects of certain essential oils namely Cinnamomum verum, Syzygium aromaticum, Cymbopogon citratus, Cymbopogon martini and their major components cinnamaldehyde, eugenol, citral and geraniol respectively, on growth, hyphal ultrastructure and virulence factors of Aspergillus fumigatus and Trichophyton rubrum. The antifungal activity of essential oils and their major constituents was in the order of cinnamaldehyde > eugenol > geraniol > C. verum > citral > S. aromaticum > C. citratus > C. martini, both in liquid and solid media against T. rubrum and A. fumigatus. Based on promising antifungal activity of eugenol and cinnamaldehyde, these oils were further tested for their inhibitory activity against ungerminated and germinated conidia in test fungi. Cinnamaldehyde was found to be more active than eugenol. To assess the possible mode of action of cinnamaldehyde, electron microscopic studies were conducted. The observations revealed multiple sites of action of cinnamaldehyde mainly on cell membranes and endomembranous structures of the fungal cell. Further, test oils were also tested for their anti-virulence activity. More than 70% reduction in elastase activity was recorded in A. fumigatus by the oils of C. verum, C. martini, eugenol, cinnamaldehyde and geraniol. Similar reduction in keratinase activity in A. niger was recorded for the oils of C. martini and geraniol. Maximum reduction (96.56%) in elastase activity was produced by cinnamaldehyde whereas; geraniol caused maximum inhibition (97.31%) of keratinase activity. Our findings highlight anti-elastase and anti-keratinase activity of above mentioned essential oils as a novel property to be exploited in controlling invasive and superficial mycoses.

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Introduction

Fungal infections caused by various pathogenic and opportunistic groups are on the rise in the different parts of the world. Invasive aspergillosis caused by Aspergillus is considered as a major cause of morbidity and mortality in immunocompromised hosts and mortality rates may range from 40 to 90% in high risk populations (Dagenais and Keller 2009). Other chronic infections associated with the immunocompromised patients are caused by dermatophytes mainly Trichophyton sp, and had shown increased incidence in recent years especially in the tropical countries (Venkatesan et al. 2007). With the increasing number of immunosuppressed patients at an unprecedented rate, the management of these fungal infections would be a definite challenge to mankind.

Current antifungal therapy for such fungal infections has been threatened by the development of drug resistant strains, host toxicity of available polyenes and fungistatic mode of action of azoles (Barker and Rogers 2006). Therefore, development of newer drugs with improved efficacy and safety or alternative mode of combating infections is needed. Recent developments in fungal genomics have provided unprecedented opportunities for identifying new antifungal drug targets and subsequently exploiting in disease control. Targeting virulence and pathogenicity are now considered as valuable anti-pathogenic approaches (Gauwerky et al. 2009). Establishment of infection by fungi depends on the host–cell interaction with complex interplay of secretion of virulence factors mainly proteases including elastinases, keratinases, gelatinases, lipases and phospholipases. These extracellular enzymes are probably essential for these organisms to degrade structural barrier and to obtain nutrient and in establishing infections (Voltan et al. 2008). Plant products traditionally being used in ethnomedicine have been expected to deliver newer antifungal compounds. Anti-fungal activities of the essential oils or their major constituents against Aspergillus and Trichophyton spp. have been reported by

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Anti-candidal activity of essential oils alone and in combination with amphotericin B or fluconazole against multi-drug resistant isolates of *Candida albicans*

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Therapy for candidiasis is becoming problematic due to the toxicities of currently available antifungal agents and the increasing prevalence of resistance among the etiologic agents. Therefore, new antifungals and alternative approaches are needed. In this study, 20 fluconazole-resistant strains of *Candida albicans* were found to have varying levels of resistance to other azoles, i.e., itraconazole (MIC of 4–128 μg/ml) and ketoconazole (2–256 μg/ml). In addition, 13 of these isolates appeared resistant to amphotericin B (32–128 μg/ml). A total of 21 plant essential oils were screened for their antifungal activity against these multi-drug resistant isolates. The oils of *Cymbopogon martini*, i.e., citral and cinnamaldehyde, exhibited strong inhibitory activity with minimum inhibitory concentrations (MIC) ranging from 90–100 μg/ml. The test oils were more effective than fluconazole and amphotericin B in inhibiting azole- and amphotericin B-resistant, as well as amphotericin B-susceptible isolates. The test oils and especially eugenol, exhibited significant synergy with fluconazole or amphotericin B against the test isolates. These findings suggest the possible effective use of certain oils alone or in combination with fluconazole or amphotericin B against multi-drug resistant isolates of *C. albicans*.

**Keywords** *Candida albicans*, drug resistance, essential oils, synergy, fluconazole, amphotericin B

**Introduction**

Fungal infections have been increasing in recent years as a consequence of the growing number of immunocompromised patients due to HIV infection, cancer chemotherapy and organ or bone marrow transplantsations [1]. In such persons, *Candida* infections are very common causing oral, vaginal and/or systemic candidiasis. Oropharyngeal candidiasis is generally frequently encountered in AIDS patients who do not have access to highly active antiretroviral therapy (HAART) [2], whereas oral candidiasis often affects cancer patients undergoing chemotherapy and/or radiotherapy [3].

Polyenes and azoles have been the antifungals of choice in the treatment of these fungal infections. However, many problems remain to be solved for most of the available antifungal drugs such as nephro- and hepato-toxicity associated with the use of amphotericin B [4]. A lipid formulation of amphotericin B is less toxic but more costly [4,5]. Azoles, particularly fluconazole, are less toxic after oral or intravenous administration and consequently are often employed [4]. However, azole therapy failures have been observed due to intrinsic resistance in *Candida* spp., such as *C. krusei* and *C. glabrata* and acquired resistance in previously susceptible strains of *C. albicans* due to their continual use in AIDS and cancer patients [6]. About 3.6–7.2% of *C. albicans* isolates from women with vaginitis are resistant to fluconazole [7] and oropharyngeal candidiasis due to the fluconazole-resistant *Candida* has long been a problem for HIV patients [8,9]. Since the immunocompromised population is increasing in number,
Antifungal activity of essential oils and their synergy with fluconazole against drug-resistant strains of Aspergillus fumigatus and Trichophyton rubrum

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Abstract The aim of this study was to screen certain plant essential oils and active compounds for antifungal activity and their in vitro interaction with fluconazole against drug-resistant pathogenic fungi. The methods employed in this work included disc diffusion, broth macrodilution, time kill methods and checkerboard microtiter tests. Oil compositions were evaluated by gas chromatography-mass spectrometry (GC-MS) analysis. Transmission electron microscopy was used to assess the effect of essential oils on cellular structures of test fungi. Test fungal strains exhibited resistance to at least two drugs (fluconazole and itraconazole). Among the 21 essential oils or active compounds tested, ten showed promising antifungal activity. GC-MS analysis revealed the presence of major active compounds in the essential oils used. Cinnamaldehyde showed the most promising antifungal activity and killing potency against Aspergillus fumigatus MTCC2550 and Trichophyton rubrum IOA-9. Cinnamaldehyde showed strongest synergy with fluconazole against A. fumigatus and T. rubrum by reducing the minimum inhibitory concentration of fluconazole up to 8-fold. Zones of lysis of the cell wall and cell membrane appeared to be where cinnamaldehyde acted on fungi. This study highlights the broad spectrum antifungal activity of essential oils and active compounds and their synergy with fluconazole against drug-resistant fungi.

Keywords Antifungal drugs · Drug resistance · Essential oils · GC-MS · Synergy · Transmission electron microscopy

Introduction

Incidence of microbial infections has increased in recent decades, especially mycoses which account for a high rate of death among patients with a weakened immune system. Opportunistic fungal infections are a serious threat to such patients and have been reported to occur at an alarming rate (Pinto et al. 2006). Infections of alveolar tissues by members of genus Aspergillus produce a spectrum of lung diseases known as aspergillosis. The disease involves pulmonary aspergillosis, invasive aspergillosis and allergic bronchopulmonary aspergillosis and is mainly caused by Aspergillus fumigatus and A. niger and less frequently by A. flavus and A. clavatus. Invasive aspergillosis in immunosuppressed individuals often results in death and has become a major concern among public health officials (Lutz et al. 2003; Singh and Paterson 2005; Erjavec et al. 2009; Leventakos et al. 2010; Pfaller and Diekema 2010). Also, infections of hair, skin and nails have increased considerably among pediatric and geriatric populations (Mukherjee et al. 2003; Monod 2008). Such infections are primarily caused by Trichophyton rubrum and other dermatophytes and are not life-threatening; however, both immunocompetent and immunosuppressed persons are affected (Vermount et al. 2008). Chronic infections of skin carry considerable morbidity and can become serious in immunocompromised patients resulting in invasive infections (Sokovic et al. 2006).

The antifungal drugs most commonly used against these diseases include amphotericin B, ketoconazole, fluconazole, terbinafine and flucytosine. Adverse side effects are associated with the use of available antifungal drugs including nephrotoxicity, hepatotoxicity and neurotoxicity (Andriole 1994). Also, therapeutic response is slow in immunocompromised patients. Fluconazole is considered to be one of the safest...
Bioactive compounds from Punica granatum, Curcuma longa and Zingiber officinale and their therapeutic potential

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Abstract
Plants have been one of the major sources of medicines since the dawn of human civilization. The contribution of plant-derived drugs in modern times is still significant and much interest has been focused on exploiting the wide diversity of medicinal plants in both traditional systems of medicine and modern drug development. In this review, we assess three plants, namely Punica granatum L., Curcuma longa L. and Zingiber officinale Rosc., for their biological activities. Recent trends in phytochemical investigation and study of the pharmacological actions of all P. granatum components (juice, seeds, leaf, pericarp) suggest possible clinical application for the treatment and prevention of cancer and other diseases where chronic inflammation is believed to play an essential etiological role. C. longa extracts and active constituents have a potential role in the prevention of cancer and the management of infectious and chronic diseases. Z. officinale extracts and active constituents have potent antioxidant, anti-inflammatory, antimutagenic and antimicrobial activities, and some of them have shown anticancer activity in experimental models of carcinogenesis. Z. officinale has also been found to be effective against pregnancy-induced and postoperative nausea and vomiting, and has proved useful for treating motion sickness and arthritis symptoms.

Introduction

Herbs, spices, medicinal plants and their preparations have been used to treat ailments since prehistoric times. The treatment of various diseases using plant-based medicines has remained an integral part of many cultures across the globe. The World Health Organization (WHO) estimates that 80% of the people living in developing countries almost exclusively use traditional medicine. Such medicines, derived directly or indirectly from plants, constitute 25% of the pharmaceutical armamentarium. India has been identified as one of the top 12 mega-diversity centers in the world, with immensely rich medicinal and aromatic plants occurring in diverse ecosystems. These medicinal plants are used both for primary healthcare and for treating chronic diseases such as AIDS, cancer, hepatic and cardiac disorders and age-related diseases such as memory loss, osteoporosis and diabetes. In the Indian codex system, Ayurveda currently uses as many as 1,003 single drugs and over 8,000 compound formulations of recognized merit. Similarly, 600-700 plants are used by other systems, such as Unani, Siddha and Amchi (1).

The use of medicinal plants and other natural products with therapeutic properties is as ancient as human civilization and for centuries natural products were the main source of drugs. About 25% of the drugs prescribed worldwide still come from plants. With 121 such compounds still currently in use. At the beginning of the 21st century, 11% of the 252 drugs considered basic and essential by the WHO were exclusively of flowering plant origin. In recent years, there has been serious thinking about the use of medicinal plants and traditional medicines as a source of new bioactive compounds for the treatment of various ailments (2-5). This is primarily due to the slow progress in drug development from synthetic and other modern approaches and the acceptance of herbal medicine in both developed and developing countries. Traditional medicinal practices form an integral part of...
4. Biofilms of *Candida albicans*, Prevented and Destroyed by Plant Essential Oils: A New Hope for Combating Drug-resistant *Candida*

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Intrinsic drug-resistance and persistent infections of *Candida* biofilms has necessitated the search for new strategies to combat such infections. In the present study, 16 out of 18 multidrug-resistant strains of *Candida albicans* formed biofilms. Preformed *Candida* biofilms showed up to 512 times increased tolerance to antifungal drugs compared to essential oils (up to 4 times). Test oils were also found more cidal in nature cidal in nature compared to antifungal drugs. At 1/2 MIC, eugenol and cinnamaldehyde were most inhibitory against biofilm formation as also evident from electron microscopic studies. Our findings suggest a novel approach to use essential oils especially eugenol and cinnamaldehyde in treating biofilm associated candidiasis.