

Phytochemicals as Antimicrobial Agents Against Opportunistic Candidiasis: A Review

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ABSTRACT

Globally, over 300 million people suffer from a serious fungal infection and about 25 million of them have high risk of dying or loss of sight. The serious fungal diseases can be either severe (such as Cryptococcal meningitis and fungal eye infection), periodic (such as *Candida vaginitis* or oral candidiasis in AIDS patients) or chronic (such as chronic pulmonary aspergillosis or fungal hair infection). *Candida albicans* is accountable for most fungal infections in humans. The vaginal yeast infection, termed as vulvovaginal candidiasis or vulvovaginitis, and oral candidiasis, a common type of yeast infection that occurs on the tongue or palate as thick white patches, are most common yeast infections found in humans. Invasive mycoses, mainly caused by *Candida*, are life-threatening opportunistic infection that has emerged as a major cause of morbidity and mortality in hospitalized patients. Despite the introduction of new and novel antifungal drugs, growing antifungal resistance is a major health concern for the clinicians and researchers across the globe. Phytochemicals represents the most abundant and extensively distributed substances in the plant kingdom. Several woody plant and herb cells produce and gather these range of medicinal phytochemicals such as phenolic compounds (hydroxybenzoic and hydroxycinnamic acids, acetophenone, and flavonoids), oligo- or polymeric compounds (such as tannins and lignins) etc. The developing antifungal drug resistance has forced the attention of researchers towards herbal products, mainly phytochemicals, in search of development of novel, safe and economically viable antifungals.

INTRODUCTION

Fungi are found almost everywhere. Around 1.5 million different species of fungi inhabits Earth, however only about 300 of these species are known to cause infections in humans.¹ Fungi live *al fresco* in moist soil, on plants and trees, on decaying vegetation and in addition they can also grow on many indoor surfaces as well as on different human body parts. Except some, most fungi are non-pathogenic. Even though fungal infections cause significant amount of human morbidity and mortality but the impact of these 'opportunistic' diseases on human health is not widely highlighted.² Due to this ignorance by global policy makers and most international health agencies, the research into the pathophysiology of human fungal infections is slow in comparison to other disease-causing pathogens. Global Action Fund for Fungal Infections (GAFFI), an international organisation working globally to reduce fungal infection cases and deaths associated with them, reported that 300 million people worldwide of all ages suffer from a serious fungal infection every year. Of these fungal infection is estimated to cause over 1.35 million deaths globally which is almost in comparison to the mortality cases estimated in malaria (1.2 million) and tuberculosis (1.4 million).^{3,4} Majority of these serious fungal infections remains 'hidden' in the patient body because of other primary health disorders such as, AIDS, cancer, transplantation, or immunosuppression etc. The common fungal diseases include Candidiasis (caused by yeasts of genus *Candida*), Aspergillosis (caused by

Aspergillus), Blastomycosis (caused by *Blastomyces dermatitidis*), Coccidioidomycosis (caused by *Coccidioides*), Fungal eye infections, Pneumocystis pneumonia (caused by *Pneumocystis jirovecii*), Ringworm (common skin infection), Sporotrichosis (caused by *Sporothrix schenckii*) etc. Fungi can be either true pathogens (such as *Coccidioides*) which cause fungal infections in healthy individuals or opportunistic pathogens (such as *Aspergillus*, *Candida*) which cause infections in immunocompromised individuals (mainly in cancer patients, organ-transplant recipients, and HIV infected persons).^{5,6} Over 20 species of *Candida* yeasts have been documented which cause infection in humans and the most common of them is diploid fungus *Candida albicans*.^{7,8} Candidiasis is one of the most common fungal infections caused by yeasts of the genus *Candida*. The species is in Kingdom: *Fungi*, Division: *Ascomycota*, Class: *Saccharomycetes*, Order: *Saccharomycetales*, Family: *Saccharomycetacea*, Genus: *Candida*. *Candida* yeasts generally grow on the skin and mucous membranes in host humans without causing any infection (that is, as harmless commensals or endosymbionts of hosts); but, overgrowth of *Candida* can cause pathogenesis. Only when mucosal barriers are interrupted or the immune system of the person is compromised, fungal cells invade and cause disease condition. Candidiasis symptoms vary depending on the body part infected.⁷

Major Types of Candidiasis

The three types of Candidiasis are Oropharyngeal/Esophageal Candidiasis (commonly referred as thrush), Genital/Vulvovaginal Candidiasis (commonly referred as vaginal yeast infections) and Invasive Candidiasis.⁷ The most common fungal pathogens isolated from the oral cavity are *Candida* species which causes oropharyngeal candidiasis (also referred as oral thrush).^{9,10} The most frequent symptom is white patches or plaques on the tongue and other oral mucous membranes (such as gastrointestinal tract or esophagus). Oral thrush infection is not very common in healthy persons and mainly affects persons with weakened immune system. Clinical prevalence of oral candidiasis among babies less than one month old, AIDS patients and cancer patients is nearly 5% to 7%, 9% to 31% and 20% respectively.⁷ Genital / vulvovaginal candidiasis (VVC; vaginal yeast infections) arises when there is an overgrowth of the normal yeast in the genital region of female vagina.¹¹ VVC is relatively common in adult women. It is estimated that nearly 75% of all adult women in their lifetime suffer from at least one 'yeast infection'.⁷ On extremely rare occasions, men could also get genital candidiasis. General symptoms of VVC in women include genital itching, burning sensation at genital, and on occasions a curd-like vaginal discharge. Symptom of genital candidiasis in men is itchy rash on the penis. *Candida* yeasts when enter the bloodstream, is commonly referred as Candidemia or Invasive candidiasis.¹² This type of bloodstream infection is extremely rare in people but common among hospitalized patients. In the general population, the Candidemia incidence is approximately 8 to 10 cases per 100,000 people. About 5% to 20% of newborn babies, weighing less than 2.2 pounds or 1000 grams, are estimated to develop the invasive candidiasis.⁷ In systemic candidiasis, immunocompromised host suffers from disseminated disease and sepsis (potentially fatal whole-body inflammation caused by severe infection).¹³

Incidence and Prevalence of Fungal Infections in India

The variable geographical and environmental conditions along with tropical and monsoon driven climatic conditions in different parts of India provide favourable conditions for the growth and development of various fungi species.¹⁴ Fungal infections can be either intrinsic (Candidemia), or extrinsic (Invasive aspergillosis or zygomycosis).^{7,15} Available data from tertiary care centers suggest that there is growing number of cases of invasive candidiasis (candidemia), aspergillosis (caused by *Aspergillus*, a common mold) and zygomycosis (caused by bread mold fungi of the *Zygomycota* phylum) infections throughout the Indian subcontinent.¹⁶ The most common opportunistic mycosis in India is invasive candidiasis.^{16,17} Candidemia, as discussed above, routinely originates as skin infection or at gastro-intestinal tract by bacterial translocation and is diagnosed by blood culture or other body fluids. The other opportunistic mycosis, invasive aspergillosis and invasive zygomycosis, are ranked as second and third respectively in India. Invasive candidiasis cases vary between 1 to 12 individual(s) per thousand patients admitted in hospitals across the country. Bloodstream candidemia is ranked fourth among the most

common infection reported across northern India region, while most of the cases are from intensive care units (ICUs).¹⁶ Mortality percentage in candidemia cases varies from 28% to 71.4%. Furthermore, a global change in varieties of *Candida* species has been observed in major hospitals across India. Interestingly higher prevalence of candidemia is caused by *Candida tropicalis* instead of *C. glabrata* or *C. parapsilosis*. The probable reason might be resistance to commonly recommended azole drugs, although it's low but it is on a slow rise over the years. Acute leukemia patients sputum and urine samples have also shown significant levels of opportunistic fungi *Candida* mainly *Candida albicans*, *C. tropicalis* and *C. pseudotropicalis*.¹⁸ Cardinal endocarditis (inflammation of the inner layer of the heart, the endocardium), hepatosplenic candidiasis (acute disseminated candidiasis in liver or spleen), fungal peritonitis (fungal infection of the peritoneum, a membrane that lines our inner abdominal wall and covers all the body organs), central nervous system (CNS) candidiasis and candidal arthritis (mycotic arthritis that affects bone and joint tissues) have been commonly reported from different hospitals across the country.¹⁶ Patients on continuous ambulatory peritoneal dialysis (CAPD) and severe pancreatitis (inflammation of the pancreas) have been found to have high risk of mortality due to candidal infections. These opportunistic fungal infections are mostly associated with haematological malignancy, organ transplant recipients, HIV, malnourishment and premature new child. Early diagnosis, timely therapy and clinical awareness are the effective strategies to limit the devastating fungal infections.¹⁹

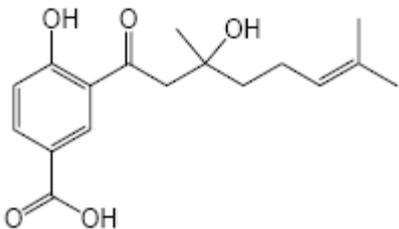
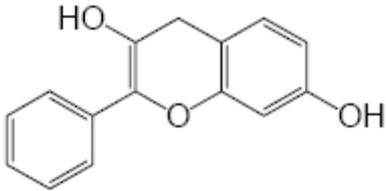
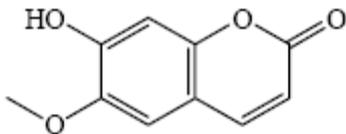
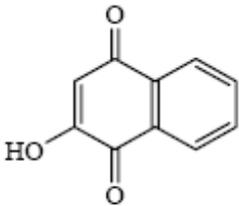
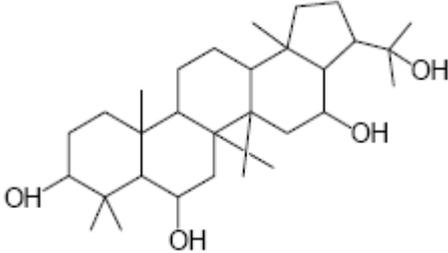
Current Antifungal Drugs

Remarkable expansion of antifungal research and development (R&D) in the last few decades has led to the discovery of novel antifungal-lipid formulations of Amphotericin B (Amphotericin B colloidal dispersion and liposomal Amphotericin B), second-generation broad spectrum triazoles (fluconazole, itraconazole, voriconazole and posaconazole) and echinocandins (casprofungin, anidulafungin and micafungin).^{19,20,21} Amphotericin B (AmB), a commonly recommended antifungal, acts by binding to ergosterol, a sterol component of the cell membrane of subjected fungi. The AmB complex forms transmembrane channels which results in change of cell permeability thus resulting in release of monovalent ions (Na^+ , K^+ , H^+ , and Cl^-) out of the cell and causing fungal cell death.²² AmBisome (liposomal amphotericin B) injections, newest in antifungal arsenal, is used for treatment of infections caused by *Aspergillus*, *Candida* and *Cryptococcus* species. This intravenous injection is a sterile, non-pyrogenic lyophilized product containing 50 mg of amphotericin B intercalated into a liposomal membrane. AmBisome[®] is officially marketed by Astellas Pharma US of Gilead Sciences, Inc.²³ Liposomes are actually closed, spherical vesicles produced by combining specific proportions of amphiphilic substances (like phospholipids and cholesterol). Subsequently the amphiphilic substances arrange themselves into single or several concentric

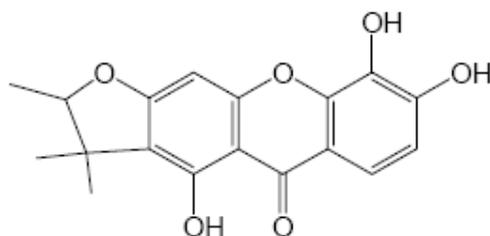
bilayer membranes once hydrated in aqueous solutions thus forming a single/multiple bilayer liposomal drug delivery system.²⁴ AmBisome is a true single bilayer liposomal drug delivery structure containing Amphotericin B drug. Some of other commonly prescribed antifungals includes azole compounds (itraconazole and fluconazole), polyenes (AmB-deoxycholate, AmB lipid complex), echinocandins (caspofungin, anidulafungin, and

micafungin) and flucytosine.²⁵ The Infectious Diseases Society of America (IDSA) Standards and Practice Guidelines Committee (SPGC) has divided the general antifungal agents for the treatment of candidiasis into four major groups: the polyenes, the triazoles, the echinocandins, and flucytosine (fluorinated pyrimidine analogue).²⁶

Table 1. Major groups of antifungal compounds derived from plants (Data compiled from: Arif T *et al.*, 2011).

Phytochemicals	Common compounds	Properties	Common plant sources
Simple phenols and phenolic acids	 <p>Crassinervic acid</p>	Simplest bioactive phenolic compounds	<i>Piper crassinervium</i> , <i>Piper aduncum</i> , <i>Piper hostmannianum</i> , <i>Piper gaudichaudianum</i>
Flavonoids	 <p>Galangin</p>	Hydroxylated phenolic substances	<i>Pelalostemium</i> , <i>Helichrysum aureonitens</i> , <i>Erythrina burtii</i> , <i>Aquilegia vulgaris</i>
Coumarins	 <p>Hydroxycoumarin scopoletin</p>	Phenolic substances consists of fused benzene and α -pyrone rings	<i>Melia azedarach</i> , <i>Mammea longifolia</i> , <i>Clausena excavate</i>
Quinones	 <p>Lawsone</p>	Aromatic rings with two ketone substitutions	<i>Rubia tinctorum</i> , <i>Rhamnus frangula</i> , <i>Hypericum perforatum</i> , <i>Hopea exalata</i> , <i>Lawsonia inermis</i>
Saponins	 <p>Mollugenol-A</p>	Glycosides with a distinctive foaming characteristic	<i>Capsicum frutescens</i> , <i>Mollugo Pentaphylla</i> , <i>Solanum chrysotrichum</i> , <i>Clematides tangutica</i> , <i>Tribulus terrestris</i>

Xanthenes



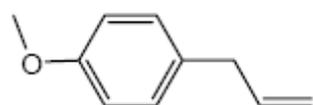
Toxyloxanthone C

Polyphenols biosynthetically related to flavonoids.

Garcinia mangostana,
Cudrania fruticosa,
Calophyllum caledonicum

Terpenes
Terpenoids

or

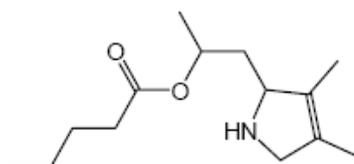


Estragole

Isoprenoids (C₁₀H₁₆) mainly diterpenes, triterpenes, tetraterpenes (C₂₀, C₃₀, and C₄₀), and sesquiterpenes (C₁₅).

Agastache rugosa,
Amaranthus viridis, *Solidago virgaurea*, *Bellis perennis*, *Ocimum gratissimum*

Alkaloids



2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate

Heterocyclic nitrogen compounds

Datura metel,
Epinetrum villosum,
Cleistopholis patens

Lectins

Potato tubers protein AFP-J and Pineapple leaf chitinase-B

Carbohydrate-binding proteins

Astragalus mongholicus,
Amaranthus,
Solanum tuberosum.

Drug Resistance in Fungi: An Emerging Problem

Although several new antimicrobial drugs have been licensed over the past decade, but increasing antimicrobial drug resistance cases is becoming a major public health concern. For example fluconazole- a commonly recommended antifungal drug- has been used to treat about 16 million patients across the globe since its launch. Due to its widespread use, there has been increasing antifungal resistance reports across the world.^{27,28} This antimicrobial resistance threatens the effective prevention, diagnostic, and therapeutic interventions due to growing range of human infections caused by bacteria, parasites, viruses and fungi. World Health Organization (2014) categorizes antimicrobial resistance as the resistance developed by the microorganism to an antimicrobial drug which was initially effective in treatment of such infections.²⁹ These global health issue are becoming an increasingly serious threat to all sectors and societies, as new resistance mechanisms by infection-causing bacteria, parasites, viruses or fungi are emerging and spreading.^{30,31} Infections caused by these resistant microorganisms often fail to respond and get killed by the standard drug regime, resulting in patient's prolonged illness, huge healthcare expenditures, and a greater risk of fatality. The burden of

antifungal resistance is becoming a major concern especially in immunocompromised patients causing significant morbidity and mortality in them. Widespread use of antifungal drugs is thought to be the major reason for development of drug-resistant *Candida* species and subsequent failure in treatment of infection.^{28,32} An insight into the mechanisms of antifungal resistance is critical for timely and efficient treatment of patients with invasive candidiasis. The increase in the drug-resistant *Candida* strains to commercial antifungals has caught the attention of clinicians and medicinal plant products (commonly referred as phytochemicals) have become a significant alternative for discovery of commercially viable, economically cheaper and safe phytomedicines.³³

Medicinal Plants: A Potential Source for Anticandidal Drugs

Over the decades, a range of human fungal infections is mounting due to augmented cases of cancer, AIDS, organ-transplantation and immunocompromised patients. Inappropriate therapeutic use of conventionally available drugs has also resulted in the development of multi-drug-resistance pathogens. Plant kingdom provides many useful lead compounds as new classes of antimicrobial agents with novel structures. These medicinal products are referred as complementary and alternative system of

medicines (CAM). Ancient Greek physician Hippocrates (in the late 15th century B.C.) cited around 300 to 400 medicinal plants.³⁴ In the first century A.D., Roman physician Pedanius Dioscorides put in writing “*De Materia Medica*”, an extensive guide to medical botany that included pharmacologically important medicinal plant catalogue. Approximately 2,50,000 to 500,000 species of plants grow on this planet and a small percentage (around 1 to 10%) of these plants act as food source for both humans and animals. The medicinal plants are now seen as an alternative to cure fungal infections. Reports suggest numerous medicinal plants are rich source of antifungal bioactive secondary metabolites (mentioned in Table 1).³⁵ Phytomedicines, botanicals as medicinal products, are major constituents of our traditional system of therapeutics. The foremost advantages of these plants extract is low cost, fewer side effects, and easily accessible to rural people. Till date more than 600 plants have been documented for their antifungal activities, but only few of them have been characterised for their active phytochemicals as antimicrobial agents.³⁶ The *in vivo* and *in vitro* antifungal studies of plants extracts or its components (phytochemicals) help to determine their effectiveness in animal models and further standardize method of extraction for subsequent studies to discover new phytomedicines. Our research group recently reported potent *in vitro* antifungal activity of plant extracts of *Piper longum* (Pippali), *Aloe vera* and *Withania somnifera* (Ashwagandha) against *Candida albicans* CAF 2-1 (WT) strain.³⁷ In the recent research on human fungal infections, phytochemicals have become a key choice for discovery of new antimicrobial agents, boosting the drug-discovery approach to find novel leads against these common and many-a-times lethal infections.

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CONFLICT OF INTEREST

Authors don't have any conflict of interest.

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