Review

Anti dermatophytic therapy - Prospects for the discovery of new drugs from natural products

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Abstract

Millions of people and animals suffer from superficial infections caused by a group of highly specialized filamentous fungi, the dermatophytes, which only infect keratinized structures. With the appearance of AIDS, the incidence of dermatophytosis has increased. Current drug therapy used for these infections is often toxic, long-term, and expensive and has limited effectiveness; therefore, the discovery of new anti dermatophytic compounds is a necessity. Natural products have been the most productive source for new drug development. This paper provides a brief review of the current literature regarding the presence of dermatophytes in immunocompromised patients, drug resistance to conventional treatments and new anti dermatophytic treatments.

Key words: dermatophytes, filamentous fungi, natural products, synthetic product.

Dermatophytosis

Millions of people and animals suffer from superficial infections caused by a group of highly specialized filamentous fungi, the dermatophytes, which only infect keratinized structures (Burmester *et al.*, 2011). The American Academy of Dermatology estimates that 10-20% of the population is affected by dermatophytes (Pfaller and Sutton, 2006). Dermatophytes are a group of parasitic fungi that live at the expense of the keratin in the skin, nails and hair. These microorganisms fall into the genera *Trichophyton*, *Microsporum* and *Epidermophyton*, as characterized according to the formation and morphology of their conidia (Weitzman and Summerbell, 1995; White *et al.*, 2008 Peres *et al.*, 2010). In general, dermatophytes are confined to the stratum corneum of the epidermis and skin appendages, especially in the moist areas of the body, such as the regions between the toes, groin and below the breasts (Chuang *et al.*, 2007). Although dermatophyte infections are restricted to areas of the epidermis, they can be invasive and cause serious widespread infections in immunocompromised patients, with the development of granulomas

(Peres *et al.*, 2010). In such cases, the disease no longer is superficial and reaches deeper layers of dermis, causing granulomatous lesions.

Dermatophytes produce proteolytic enzymes, keratinases, which are able to hydrolyze keratin, the main protein constituent of hair, nails and skin. The infection can be mild to severe, depending on the host immune response (Akcaglar *et al.*, 2011). The keratin, collagen and elastin constitute 25% of the mass of mammals. The enzyme required to hydrolyze these macromolecules is found in infected tissues and is therefore considered essential to the virulence of dermatophytes (Simpanya, 2000). Colonization by a dermatophyte, and its ability to cause an infection in the host, depends on several factors, among which are the "escape" mechanisms of the host resistance, including dry skin, a slightly acidic pH, the continuous regeneration skin, the fungicidal effect of fatty acids, the state of the keratinized layer and other factors, such as competition with the normal skin microbiota (Erbagci *et al.*, 2004). The establishment of the infection is initiated by the inoculation of arthrospores deposited on the skin, favored by a pre - exist-

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ing skin lesion or abrasion (Sidrim and Rocha, 2004) and the microorganism's remarkable enzymatic ability to degrade keratin (Simpanya, 2000; Abdel-Rahman, 2001; Macêdo *et al.*, 2005) . It also can infect several animal species, creating generally dry, rounded and usually non-pruritic lesions, distributed focally on the skin without causing a general inconvenience to the affected animals (Pereira and Meireles, 2001; Sobestiansky, 2001) . The animals serve as reservoirs of zoophilic dermatophytes and their zoonotic infections have considerable importance (Cabañes, 2000; Baranova *et al.*, 2003) . Anthropophilic species are not part of the normal microbiota of humans nor are these microorganisms opportunistic, but true pathogens that infect keratinized tissues, nails or hair of healthy individuals (Baeza *et al.*, 2007; Monod, 2008). Dermatophytes digest keratin and have the ability to invade living tissues and cause certain injuries (Simpanya, 2000), with the severity of the infection being related to, in part, the reaction of the host to the invading organism, and other factors, such as species or virulence of the infecting strain, the host reaction to the metabolic products produced by the fungus, anatomic site of infection, and local environmental factors.

Treatment of Mycosis

Forsuccessful of infection by dermatophytes, the pathogen required to overcome the defense mechanisms of the host. A important mechanism of host defense against these pathogens is the keratinization which is the process of epithelial desquamation leading to removal of the fungus (Peres *et al.*, 2010). For treating the fungal infections of skin, topical medications are appropriate only for early or mild infections, especially those caused by *T. rubrum*, the main cause of *Tinea pedis*. In nail infections (onychomycosis) and infections caused by zoophilic dermatophytes, mainly leading to the development of *Tinea capitis* and corporis, the usual therapy is systemic (Tani *et al.*, 2007; White *et al.*, 2008). Terbinafine inhibits the growth of dermatophytes of all genera and is the main drug of choice for the treatment of dermatophytoses, especially with chronic conditions. The naftifin and terbinafine represent the group of drugs synthetic, the allylamine, which were described as drugs in clinical treatment of superficial mycoses (Balfour and Faulds, 1992). It has shown fungicidal activity against a wide range of dermatophytes, molds and certain fungi dimorphic, and fungistatic activity against *Candida albicans* being that terbinafine has been the drug of first choice for dermatophytes (Abdel-Rahman and Nahata, 1997; McClellan *et al.*, 1999). Its mechanism of action relies on blocking the biosynthesis of ergosterol, an essential component of fungal cell membranes, through inhibition of fungal squalene epoxidase (Abdel-Rahman and Nahata, 1997). The tolerability of these drugs is generally high after topical administration or oral. In comparative studies, the incidence of adverse effects associated with use of terbinafine orally, was lower than that observed with

griseofulvin and similar to itraconazole (Abdel-Rahman and Nahata, 1997). Terbinafine has emerged as a new therapeutic option for dermatophytoses in both humans and animals (Mancianti *et al.*, 1999). Griseofulvin is used exclusively to control the development of keratinized tissue infection by presenting only fungistatic and not fungicidal action. This drug is orally administered and treatment varies according to the clinical form of mycosis (Gupta *et al.*, 2001). In severe infections, the treatment must be prolonged (Deacon, 1998). The mechanism of action of this drug is associated with interfering with the polymerization of microtubules causing abnormalities in cell division due to achromatic spindle formation and abnormal growth, probably due to disruption of intracellular transport associated with microtubules (Odds, 2003). Currently, imidazole derivatives represent a major advance in oral and topical treatment of superficial mycoses, with dermatophytosis specifically, clotrimazole (topical), ketoconazole, itraconazole and fluconazole are commonly used because they are absorbed through the gastrointestinal tract and maintain an effective activity (Katzung *et al.*, 1998), with fluconazole being effective against other types of fungal infections (Follador *et al.*, 2001; Oliveira *et al.*, 2002). In therapy, there are two groups of antifungal azoles are used in the clinic: the imidazoles (ketoconazole, miconazole, clotrimazole and econazole) and triazoles (fluconazole, itraconazole, voriconazole and posaconazole). The use of imidazoles is limited to the treatment of superficial mycoses. Triazoles have a larger application in these therapies. In dermatology, chronic infection is treated with severe drugs such as terbinafine, itraconazole and fluconazole, which are the most used (Abdel-Rahman *et al.*, 1998; Fernández-Torres *et al.*, 2001; Fernández-Torres *et al.*, 2003). In an attempt to increase the cure rate of drug treatments, combined topical and oral anti-inflammatory drugs have been used. There have recently been cases of treating onychomycosis using topical amorolfine and ciclopirox (Gupta *et al.*, 2001).

Resistance to Drugs

There is a large arsenal of antifungal drugs, however, their cellular targets are limited because of the similarity that exists between fungi and host cells. Drugs that are commonly used are directed against the biosynthetic pathway (Martinez-Rossi *et al.*, 2008). The reports of recurrences are usually associated with the discontinuation of therapy, although clinical resistance to the antifungal drug terbinafine has been described in the literature (Mukherjee *et al.*, 2003) described a case of clinical resistance to this drug. The strain studied was taken from a patient with onychomycosis was that treated with oral terbinafine, which was not effective with the microorganism presenting cross-resistance to several other drugs, including naftina, butenafine, and tonoftato tolciclato, suggesting a target-specific resistance mechanism. The report regarding their resistance

to terbinafine has been discussed and refers to the squalene epoxidase gene mutation leading to the substitution of amino acid L393F (Osborne *et al.*, 2006). Terbinafine resistance in mutants of the *Aspergillus* species was also mentioned, and this indicated that there was a mutation in the gene encoding squalene epoxidase enzine (ErgA), resulting in high resistance to this antifungal (Rocha *et al.*, 2006; Espinel-Ingroff, 2008). In studies on the mechanisms of resistance to the fungus *T. rubrum*, two ABC-type transporters, TruMDR1 and TruMDR2, were important in the process of resistance (Fachin *et al.*, 2006; Maranhão *et al.*, 2009). The clinical resistance to griseofulvin, or relapses after treatment, is common in dermatophytosis. In a study conducted by Zomorodian, *et al.* (2007), a decreased expression of the gene encoding beta-tubulin was observed for the fungus *T. rubrum* during drug administration, with the decrease being concentration dependent. This information contributed to a better understanding of the mechanism of action and therefore can assist in more rational use of pharmacological therapy. The *in vitro* resistance of an isolate can be classified as either intrinsic or acquired. Intrinsic resistance allows all normal members of a species to tolerate a particular drug. Acquired resistance is a term used when a resistant strain emerges from a population that was previously drug-sensitive (Kamai *et al.*, 2004) . The biochemical mechanisms may contribute to the phenotype of drug resistance in fungus involve a decrease in drug uptake, structural alterations in the target site and an increase in drug efflux or in intracellular target levels, gene amplification, gene transfer, gene deletion, point mutations, loss of cis - and trans-acting regulatory elements and transcriptional activation (Kamai *et al.*, 2004) . Few studies have reported mechanisms of drug resistance in dermatophytes, although more studies are required to determine the mechanisms by which resistance occurs for these pathogens.

Natural and Synthetic Products

The search for plants with healing powers for various diseases dates back many years. From prehistory to the present day, thousands of plants have been used by people of all continents, either in the form of poultices, infusions, decoctions, and others. In the 90's, approximately ? of the American population had already made use of at least one type of unconventional therapy (Eisenberg *et al.*, 1993). In this context, interest in plants with therapeutic properties including those with antimicrobial activity has grown, not just form themselves into an alternative therapeutic approach, but also because the prospect of isolating substances with significant efficacy and a lower index of disadvantage (Queiroz *et al.*, 2009). Plants have been used in medicine for a long time, having been extensively used in folk medicine because they represent an economical alternative, are easy to obtain and are applicable to various pathologies (Rojas *et al.*, 2006). Plants are an important source of biologically active compounds, many of which are models for the synthesis of a large number of drugs (Yunes and Calixto, 2001; Simões *et al.*, 2003). Special attention has been directed to natural derivatives, based on the knowledge of antifungal compound production in nature (Wojtaszek, 1997; Gurgel *et al.*, 2005).

Natural products have served as a research resource for most drugs, providing a basis for chemical research and discovery of new drugs (Chin *et al.*, 2006). Several reasons have been offered to explain the success of natural products, among them is their great chemical diversity, the effects of evolutionary pressure in creating biologically active molecules, the structural similarity of protein targets in different species, among others (Harvey, 2007). Natural products are a source of numerous agents of therapeutic value. They also inspired, at various levels, the development and manufacture of synthetic agents of therapeutic importance. These agents are synthesized by manipulating the natural product, demonstrating that the synthesis of molecules can be used to discover and develop novel therapeutic agents (Wilson and Danishefsky, 2006). An ideal antifungal drug should have a broad spectrum of fungicidal activity and not cause toxicity to the host (Carrillo-Muñoz *et al.*, 2006). A large number of antifungal drugs have some connection with natural products. The class of polyenes and griseofulvin are natural products, whereas echinocandins are semisynthetic derivatives of natural products (Butler, 2005). Currently, antibiotics and antifungals represent a small group of drugs which plays an important role in fungal disease control, however, some of these antifungals have serious drawbacks such as toxicity, fungistatic activity, limited spectrum of action or resistance (García-Sosa *et al.*, 2011). The great importance of natural products in developing new therapeutic tools is evident. In this aspect, medicinal plants and their derivatives are important for pharmacological research and drug development. These natural products can be used directly as therapeutic agents, as well as a source of raw materials for synthesis, or can serve as prototypes for new pharmacologically active models (Brazil, 2006).

Fructus Psoraleae and *Folium Eucalypti Globuli* have long been used as Chinese medicines to treat dermatomycosis. Both pure compounds effectively inhibit the *in vitro* growth of *T. mentagrophytes* and *T. rubrum* (Lau *et al.*, 2010). In the study of Eisenberg *et al.* (1993) a volatile extract of *Eryngium duriaei subsp. juresianum*, presents an antifungal activity (Minimal inhibitory concentration (MIC) values = 0.16-0.32 µL.mL (-1)) against several dermatophyte species (*T. mentagrophytes, T. rubrum, E. floccosum; T. verrucosum, T. mentagrophytes var. interdigitale, M. canis* and *M. gypseum*) (Eisenberg *et al.*, 2010). Machado *et al.* (2009) evaluated the antifungal activity of *Eugenia umbelliflora Berg.* against some dermatophytes (*E. floccosum, M. canis, M. gypseum, T. rubrum, T. mentagrophytes*) and obtained good results, with MIC values between 200 and 1000 microg/mL, and in-

terestingly, inhibited 4/5 species with MIC values of < or = 500 microg/mL. Koroishi *et al.* (2008) studied the *Piper regnelli* extract, with the results indicating that this plant had strong activity against *T. mentagrophytes, T. rubrum, M. canis and M. gypseum.* Park *et al.* (2011) found good results against various pathogenic fungi (species dermatophytes) with the polyphenol Epigallocatechin 3 - O-Gallate (Table 1). The antifungal activities of the essential oils (EOs) of *Acantholippia seriphioides*, *Artemisia mendozana*, *Gymnophyton polycephalum*, *Satureja parvifolia*, *Tagetes mendocina*, and *Lippia integrifolia*, collected in the Central Andes area, province of San Juan, Argentina, were investigated against *M. gypseum*, *T. mentagrophytes,* and *T. rubrum* and were inhibited by the EOs of *G. polycephalum*, *L. integrifolia*, and *S. parvifolia*, with minimum inhibitory concentrations (MICs) between 31.2 and 1000 µg/ml. This study shows that these Andean species might be used to treat superficial fungal infections (Lima *et al.*, 2011). Ponnusamy *et al.*, 2010 investigated the medicinal plant *Wrightia tinctoria* activity against dermatophytic and non-dermatophytic fungi. Leaf chloroform extract showed activity at 0.5 mg/mL against *T. rubrum*, *E. floccosum*, *Aspergillus niger* and *Scopulariopsis brevicaulis*. The major compound, identified as indirubin, exhibited activity against dermatophytes such as *E. floccosum* (MIC = 6.25 µg/mL); *T. rubrum* and *T. tonsurans* (MIC = 25 µg/mL); *T. mentagrophytes* and *T. simii* (MIC = 50 µg/mL). It was also active against non-dermatophytes (*Aspergillus niger*, *Candida albicans* and *Cryptococcus* sp.) within a MIC range of 0.75-25 µg/mL. All of the new chemical entities approved as drugs between 1981-2006 total almost 1,184 products, with 5% as natural products, 47% as semi-synthetic derivatives of natural products, mimicking natural products and products synthe-

sized with pharmacological groups based on natural products, 18% as by-products and vaccines and 30% as fully synthetic products (Newman and Cragg, 2007). The originality of many structures of natural products attracts attention to their use as a starting point for semi-synthesis and total synthesis (Butler, 2005). Soares *et al.* (2010) used semi-synthetic esters of protocatechuic acid isolated from *Cupania oblongifolia* against *T. rubrum* and *T. mentagrophytes,* demonstrating an antifungal activity with MIC values lower than 31.25 ug/mL. The modification of natural products by semi-synthesis has led to different types of drug combinations. The development of chemotherapeutic agents of synthetic origin and the discovery of powerful new antimicrobians isolated from natural sources account for invaluable contributions in the fight against bacterial and fungal resistance (Silveira *et al.*, 2006). Pisseri *et al.* (2009) conducted a randomized open clinical trial on 60 thorough breeding horses affected by equine ringworm using Tea Tree Oil (TTO). The animals were randomly divided into 2 groups of 30 subjects. Diagnostic criteria were the presence of clinical signs and positive *Trichophyton equinum* culture. Specificity control using TTO mixture in 5 not dermatophyte affected animals was achieved also. The antimycotic activity against *T. equinum* of a mixture containing 25% TTO in sweet almond oil, was evaluated in vivo treating 30 subjects, the others were administered enilconazole 2% solution. The animals of both groups were topically treated twice a day for 15 days with a 25% mixture of TTO diluted in sweet almond oil and every 3 days, four times with enilconazole rinses, respectively. All the treated animals showed complete clinical and a etiological healing. Part of control subjects also, showed an improvement and none of them exacerbate the lesions. The authors believe this TTO an alternative for practitioners interested in herbal medicines, contributing to fulfill the gap existing between

Table 1 - Antifungal activity of natural products against a variety dermatophytes.

Natural Products	Microorganisms	Reference
Citrus bergamia (Rutaceae)	*T. rubrum, T. mentagrophytes T. tonsurans, M canis, M. gypseum E. floccosum*	Sanguinetti *et al.*, 2007
Brazilian Copaíba Oil (Fabaceae)	*T. rubrum, M. Canis*	Santos *et al.*, 2008
Piper regnellii (Piperaceae)	*M. canis, M. gypseum, T. rubrum, T. mentagrophytes*	Koroishi *et al.*, 2008
Syzygium aromaticum (Myrtaceae)	*M. canis, M. gypseum, T. rubrum, T. mentagrophytes, E. floccusum*	Pinto *et al.*, 2009
Eugenia umbelliflora Berg. (Myrtaceae)	*M. canis, M.gypseum, T.rubrum, T.mentagrophytes, E.floccusum.*	Machado *et al.*, 2009
Fructus Psoraleae (Leguminosae)	*T.rubrum, T. mentagrophytes*	Lau *et al.*, 2010
Protocatechuic acid (Cupania oblongifolia) (Sapindaceae)	*T.rubrum, T. mentagrophytes*	Soares *et al.*, 2010
Folium Eucalypti Globuli (Leptospermaceae)	*T.rubrum, T. mentagrophytes*	Lau *et al.*, 2010
Eryngium duriaei subsp. Juresianum (Umbelliferae)	*T. mentagrophytes, T. rubrum, E. floccosum, T. verrucosum, T. mentagrophytes var interdigitale, M. canis and M. Gypseum*	Cavaleiro *et al.*, 2011
Xanthones (Clusiaceae)	*M. canis, M. gypseum, T. rubrum, T. mentagrophytes, E. floccusum*	Pinto *et al.*, 2011
Epigallocatechin 3 – O- Gallate (green tea) (Theaceae)	*M. canis, T.rubrum, T.mentagrophytes*	Park *et al.*, 2011

in vitro and clinical studies. The extracts obtained from plants, such *Euphorbia prostata, Salvia Texana, Colubrina greggii, Clematis drummondii*, among others, have shown promising results for stimulating the search for new potential antifungal plant sources (Alanís-Garza *et al.*, 2007). Most antifungal drugs have a connection to natural products, it can be seen the potential antimicrobial plant products, and consequently, the real possibility of application of these products in the prevention and treatment of infectious diseases of fungal origin. However, it is necessary to mention the need for toxicological studies and clinical and security support for the use of these products as drugs.

Conclusion

Millions of people and animals suffer from superficial infections caused by a group of highly specialized filamentous fungi, the dermatophytes. Interest in medicinal plants has increased, especially in Brazil, due to the fact of the vast diversity of plants with therapeutic potential. It is necessary to search for new drugs because of the increase of resistant isolates. More studies are needed to develop drugs for dermatophytosis, since the conventional drugs used for this pathology, while being cytotoxic, often lead to clinical resistance.

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