Conventional and natural compounds for the treatment of dermatophytosis

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Abstract

Dermatophytes are a group of pathogenic fungi that exclusively infect the stratum corneum of the skin, nails and hair, causing dermatophytosis. Superficial skin infections caused by dermatophytes have increased in the last decades. There are conventional antifungals that treat these infections, such as terbinafine, fluconazole, and others. However, the limitations of these treatments (resistance, side effects and toxicity) along with the increasing over-prescription, the misuse of these antifungals and the high treatment costs led to the search for new, alternative, natural-based antifungal drugs. These have multiple mechanisms of action, which works to their advantage, making it difficult for a fungus to create resistance mechanisms against all of them at the same time.

The main objective of this work is to provide a state-of-the-art review on dermatophytes, dermatophytosis and the existing treatments, both conventional and natural such as, chitosan and essential oils.

Keywords: dermatophytes, dermatophytosis, antifungals, chitosan, essential oils

Dermatophytes

Dermatophytes are one of the oldest groups of microorganisms recognized as agents of human disease.[1] They are fungi with the ability to invade keratinized tissues of humans and other animals (a rare property in the fungal kingdom) and produce an infection called dermatophytosis.[2] All dermatophyte species belong to the *Arthrodermataceae* family.[1]

Dermatophytes are studied since 1841, when David Gruby discovered the fungal nature of skin infections.[3] Between 1840 and 1875, *Microsporum audouinii*, *Epidermophyton floccosum*, *Trichophyton schoenleinii*, *T. tonsurans* and *T. mentagrophytes* were already described. After Pasteur's invention of axenic culture, culturing and description of new species expanded. At that time, dermatophyte species definition was performed through the combination of clinical pictures and morphological characters *in vitro*. Between 1870 and 1920, 16 species of these fungi associated with humans were described. And, in the following decades, the number of new species and recombined names increased. This led to huge classification confusion around the year 1950. After that, the nomenclature of dermatophytes stabilized with the acceptance of three genera *Epidermophyton*, *Microscoporum* and *Tricophyton.*[1]

However, this classification in three genera is solely based on the phenotype of the species and led to the misclassification of morphological mutants that were described in separated taxa. Furthermore, there are several dermatophytes that do not sporulate in culture (or sporulate poorly) and thus present limited phenotypic characteristics.[1]

In the final decades of the twentieth century, it became clear that morphology could not be used as sole characteristic for classification or identification because of its limitations. In order to overcome these issues, Weitzman et al. (1983)[4] began studying physiological parameters, such as the ability of strains to assimilate a panel of essential vitamins (through fungal culture on trichophyton-agar), growth temperature, gelatin liquefaction, etc.

In 1961, Dawson and Gentles[5] discovered the teleomorphs of dermatophytes. Several

geophilic and zoophilic species were found to produce sexual states, and the genera *Arthroderma* and *Nannizzia* were introduced. This led to the introduction of dual nomenclature of dermatophytes. During the decades of dual nomenclature, species could have two names, but since 2013 the name, anamorph or teleomorph, always refers to the same, original type specimen.[1]

In 2017, de Hoog et al.[1] constructed a phylogenetic tree using ITS rDNA region because this gene was comparable and alignable over the entire set of strains they studied. They divided the dermatophytes into seven clades: *Trichophyton, Epidermophyton*, *Nannizzia, Paraphyton, Lophophyton, Microsporum* and *Arthroderma.* The main characteristics of dermatophytes are listed in Table 1.

So far, 16 *Trichophyton*, 1 *Epidermophyton*, 9 *Nannizzia,* 3 *Paraphyton,* 1 *Lophophyton,* 3 *Microsporum* and 21 *Arthroderma* species have been described. All of them are listed in the table below (table 2).

Ecology

In the course of evolution, dermatophytes have developed host specificity. It is thought that these fungi, initially, lived in the soil as saprophytic, but due to the increasing interactions with animals, they evolved to a parasitic lifestyle.[6] Based on their host specificity, these fungi are classified into three ecological groups: geophilic, zoophilic and anthropophilic.

Geophilic dermatophytes are usually saprophytic and obtain nutrients from keratinous substrates. These fungi rarely cause infection in animals and humans but may be carried by animals in their fur.[1,7] Zoophiles live in close association with animals and they can infect humans. They are pathogens with only one animal host and grow as saprophytes on animal materials.[1,7] These fungi occur in the fur of particular animal hosts, either symptomatically or asymptomatically, and can become epidemic.[1] When zoophilic and geophilic species are transmitted to humans, they cause acute, inflammatory mycoses. Sometimes, humans infected by zoophilic dermatophytes remain contagious,

leading to small, self-limiting outbreaks. On the other hand, most infections caused by geophilic are quickly resolved.[1]

Anthropophilic dermatophytes infect human beings, as they are their primary host, but they also can cause infection in animals. Transmission of the infection is usually from man to man.[7] They usually cause chronic, mild, non-inflammatory infections that often reach epidemic proportions.[1]

The biological function of dermatophytes in the soil is the degradation of keratinized substrates (hides, furs, claws, nails and horns of dead animals). In the soil, these fungi live in their sexual stages (teleomorph), whereas in keratinized materials, they live in an asexual stage (anamorph).[6]

The passage from the saprophytic way of life in the soil to an almost exclusively human parasitism came with a decrease or loss of conidial formation as well as failure of sexual reproduction. Along with the loss of the ability to reproduce sexually, anthropophilic species tend to produce chronic infections that are difficult to resolve spontaneously.[8]

Dermatophytosis

Infections caused by dermatophytes are called several names such as, dermatophytosis, ringworm or *tinea*, and they are infections of the skin, hair and nails caused as a result of colonization of keratinized tissues of the body.[7] Dermatophytosis are cosmopolitan, contagious mycoses that infect a wide range of mammals (including man) and more rarely birds. Dermatophytes that infect animals are mostly zoophilic, but can also be geophilic and exceptionally anthropophilic.[9] Although they can occur on people of any age, some people are more prone to be infected by dermatophytes. Factors that contribute to the development of dermatophytosis are: ownership of companion animals, use of public sports facilities, wearing occlusive training shoes, Diabetes Mellitus, vascular diseases (such as arteriosclerosis) and an ageing population. There are also some risk factors associated with the high incidence of dermatophytosis such as familiar disposition, male gender, foot trauma and

smoking.[10] Among the *Tinea* infections, *Tinea corporis*, *Tinea cruris*, *Tinea pedis* and onychomycosis are the most predominant types.[7] There are, also, geographic patterns concerning these infections: *Tinea pedis* is more common in developed countries, while *Tinea capitis* is more common in developing countries.[11]

Types of tinea

There are different types of *tinea* and their names are given according to the site of infection. Table 3 lists the types of *tinea*, the area of the body affected in each one, some characteristics/ symptoms of these conditions and the responsible fungi.

Infection of keratinized areas

Dermatophytes are not part of the normal human skin microbiota, but they are well adapted to this infection site because they can use keratin as a source of nutrients, unlike other fungal pathogens.[13] They enter the organism possibly through injured skin, scars and burns. The infection is caused by arthrospores or conidia. The fungus invades the uppermost, non-living, keratinized layer of the skin – stratum corneum, and produces the exo-enzyme keratinase, inducing an inflammatory reaction at the site of the infection.[7]

The first step is the entrance of fungal elements capable of germination into the skin or at least adherence of such elements to the stratum corneum. When vital fungal elements attach long enough to the stratum corneum, they germinate, hyphae develop and they spread centrifugally.[14] Dermatophytes release several (extracellular) enzymes during growth. These enzymes allow them to degrade and utilize keratins, other proteins, lipids and DNA as nutrient sources supporting them in the infection process.[14] Some of more relevant enzymes are described below.

Enzymes

Proteases. Proteases are one type of relevant extracellular enzymes produced by these fungi. Proteases are all enzymes that catalyze the cleavage of peptic bonds of proteins, digesting them into peptides or free amino acids. They can be divided into endoproteases and exoproteases. The first ones cleave peptide bonds within a polypeptide and the second ones cleave polypeptides only at the N- or the C-terminus of the chain.[15] Dermatophytes possess a genome encoding a battery of secreted proteases similar to that of *Aspergillus* species[15] because all dermatophyte species and *Aspergillus* belong to the same phylum, Ascomycota.[16] However, in these fungi, the genes encoding secreted endoproteases have expanded.[3]

Proteases are released from the mycelium and they can hydrolyze many soluble proteins. Some proteases are secreted on culture medium constitutively, particularly when growth begins. However, in the exponential phase, a decrease in protease production is verified due to the lack of carbon, nitrogen and sulphur sources. Extracellular proteases of dermatophytes are neutral or alkaline proteases. Their optimum pH values are in the range of 6 to 9.[17] A clear positive correlation between proteolytic activity and virulence has not yet been found in dermatophytes. This means that, in these fungi, the proteolytic and keratinolytic activities aren´t lower in opportunistic species when compared to obligate parasites. However, there are differences in proteolytic activity among strains isolated from different types of lesions.[17]

Dermatophytes also secrete multiple serine and metallo-endoproteaes called subtilisins and fungalysins, respectively. These enzymes are also known as keratinases.[18] Viani et al.[19] in 2001 established a direct relationship between keratinases and pathogenicity.

Proteolytic enzymes, especially keratinases, are partly responsible for dermatohytes' ability to invade skin and disseminate through the stratum corneum. Because of this, they have been the most studied enzymes.[20] One of the most used methods to study keratinases is to determine the keratinolytic activity of these enzymes by measuring the release of soluble proteins, peptides and amino acids from keratinaceous substrates.[17] Another method consists in measuring the clear zones around fungal

colonies grown on media with keratin.[20,21]

In this group of fungi, twelve members encoding subtilisins were registered, five members encoding secreted deuterolysins and five members encoding secreted fungalysins. At neutral or alkaline pH, dermatophytes secrete subtilisins Sub3 and Sub4 and fungalysins, Mep3 and Mep4, as endopeptidases. [3]

Dermatophyte fungalysins are glycoproteins with a molecular mass of 40-80 KDa.[15] Proteases are released from the fungal mycelium on most culture media.[17] However, they are produced at high levels when the available carbon and nitrogen sources consist of complex proteins instead of glucose or peptidic digests.[18]

Additionally, dermatophytes secrete aminopeptidases including leucine aminopeptidases (Lap1 and Lap2) and dipeptidyl-peptidases (DppIV and DppV), which showed similar activities to *A. fumigatus* orthologues.[22] It was also discovered that dermatophytes are able secrete a carboxypeptidase that is homologous to the human pancreatic carboxypeptidases.[23]

It was also reported that in a proteic medium at acidic pH, these fungi secrete an aspartic protease of the pepsin family (Pep1) and exoproteases which are tripeptidyl peptidases of the sedolisin family (Seds), prolyl peptidases and carboxypeptidases.[24]

LysM proteins. These have a role in protecting dermatophytes from host immune detection. These proteins seem to bind and mask cell wall components and carbohydrates, thereby avoiding the host's immune response to the fungi.[16,25] The importance of these proteins is supported by observations that, during infection, defective or absent cell-mediated immunity predispose the host to chronic or recurrent infections.[26]

Lipases and esterases. Dermatophytes can produce lipases and esterases and this production was demonstrated by plate tests, diagnostic kits and on liquid media. Lipase and esterase

production are usually studied in media containing lipids, but they are not only produced in these media. They are also produced in media with keratin and even in Sabouraud glucose-peptone broth. There are usually great differences in lipolytic activity between species and strains of dermatophytes. However, the lipolytic activity is usually moderate to weak.[17] For instance, *T. rubrum* has a weak lipolytic activity whereas *M. canis* demonstrates high activity.[27,28]

Elastase. Elastase is an extracellular enzyme that degrades elastin.[29] Elastin is a highly elastic protein present in connective tissues and allows them to resume their shape after stretching or contracting. In dermatophytes, elastase production has been associated with acute lesions. The elastase activity present in *T. mentagrophytes*, *T. verrucosum* and *M. gypseum*, and absent in *M. canis* suggests that the first three species are more virulent than the last ones.[19]

Other enzymes. Dermatophytes produce an extracellular phosphatase with wide specificity and optimum pH around 8.7. The enzyme is secreted constitutively because it was found in media containing inorganic phosphate.[17] The production of phosphatase occurs in *T. rubrum, T. mentagrophytes, E. floccosum* and *M. canis* has described in the literature. [17,30,31]

These fungi also produce amylase and some authors have argued that starch utilization induces amylase production. Dermatophytes can't hydrolyze pectin, native cellulose and its derivatives and products. However, they are able to hydrolyze simple sugars.[17] The production of amylase was detected in *T. mentagrophytes*.[32]

These fungi also produce kinases, including pseudokinases. These enzymes are involved in signalling and are necessary for adapting to the skin's niche.[16] Some authors described the production of kinases in *T. equinum*.[33]

Enzymes are not the only tools that allow dermatophytes to degrade keratinized subtracts. By excreting large quantities of sulphite - sulphitolysis - dermatophytes can cleave disulphide bridges.

By this process, reduced proteins become accessible for further digestion by proteases.[15]

During skin invasion, dermatophytes are not limited to the ability of producing enzymes or to excrete sulphites. These fungi are able to invade the stratum corneum due to a number of conditions in the skin that favor their growth, namely: a) the stratum corneum is an avascular tissue composed of highly specialized but dead cells. So, it is distant from the body's mains defensive mechanisms; b) the stratum corneum is well hydrated so, the skin temperature is cooler than body temperature, pH ranges from 5.5 to 6.7, and skin is exposed to the aerobic conditions of the atmosphere; c) the stratum corneum is favorable to dermatophyte growth because it is composed of proteins, amino acids, lipids, carbohydrates and various trace elements, including iron; d) in some areas of the stratum corneum there are certain anatomical considerations which may enhance the establishment of dermatophytes: hair on scalp may act as a trapping device for an airborne dermatophyte infection; the hyponychial horny layer is covered by the distal portion of the nail plate and a groove is thus constructed, which may also act as a trapping device for dermatophyte infective particles, the interdigital spaces of the toes and the crural areas in males are naturally occluded, which may explain the fact that *tinea pedis* in most instances starts in the toes webs and *tinea cruris* is almost exclusively a male disease.[34] The mechanism through which keratinophilic fungi degrade keratin is a result of the mechanical action of the fungus and the enzymatic activity of intra cellular keratinases.[35]

Dermatophytes induce the normal signs of inflammatory reactions such as redness, induration (swelling), heat and alopecia (hair loss). Inflammation causes the pathogen to move away from the infection's site and fixate at a new site. This movement of the fungus produces the classical ringed lesion.[7] There are some factors that can predispose to chronic dermatophytosis. These factors include collagen vascular disease, corticosteroid administration, diabetes mellitus, hematological malignancy, atopy and old age. It has also been documented and inherited tendency to develop chronic infection linked to an autosomal recessive trait.[36]

Impact and epidemiology of dermatophytosis

Dermatophytosis appears commonly in pet animals, in livestock and sometimes in wildlife. Because it is a contagious disease, there is a high occurrence of *tinea* in herds and animal collectivizes. Although, it is not a serious illness, it has serious economic consequences due to the long duration of the disease, the easy contamination and spread of infection among animals and the difficulties and costs of control measures. Because *tinea* is a contagious disease and due to unaesthetic aspect of the lesions, it is a limitation to the attendance at pet exhibitions and sport activities for horses, to commercial transactions and animal commerce. Furthermore, *tinea* also leads to losses in the hide and skin industry as scars resultants from *tinea* lesions reappear on leather at tawing and tannery.[9]

Animal dermatophytosis are of great concern in public health as the majority of dermatophytes isolated from animals are zoonotic.[9] These dermatophytes are the most common cause of fungal infections worldwide, affecting millions of people annually. In the United States alone, they have an economic impact on the health care system that exceeds $400 million a year.[37]

All genera of dermatophytes have a worldwide distribution, excluding Antarctica.[7,11] The distribution of dermatophytes presents a great variance around the world. Seebacher et al.[38] (2008) reported that in Europe, particularly, in Mediterranean countries and in Slovenia, the incidence of *M. canis* infections has increased. *T. rubrum* is the main cause of *tinea pedis*, nail infections, *tinea cruris* and *tinea corporis* in the world. These fungi are replacing other dermatophytes. This fact is probably related to the many possibilities of infection by *T. rubrum* due to its large distribution among the world population.

In Portugal, it is estimated that 1,510,391 inhabitants have a skin fungal infection, corresponding to an incidence of 14,3%.[39] In a study carried out between 1983 and 2002, 10,003 samples were analysed and the frequency of dermatophytes was 23.6%, with a prevalence of *tinea capitis* (4.9%).[40] In a more recent study, performed by the Portuguese National Institute of Health, the average frequency of dermatophyte infection was 21%; *Tinea capitis* was confirmed in 28% of

the patients and incidence in children (1–9 years)[41] and it was the most frequent.

Superficial skin infections caused by dermatophytes have increased during the last decades, especially among high-risk patients. Currently, the prevalence of these infections has increased and the percentage of people infected with skin mycoses is more than 20%.[42] Although in developed countries the incidence of *tinea capitis* is decreasing, *tinea pedis* and onychomycosis are becoming an epidemiologic and economic problem.[38] In 1998, Svejgaard[43] listed the three main factors for the distribution of dermatophytes in Europe. These factors are: 1) the existence of a poor living standard in several Eastern and Southern European countries is responsible for the increase in zoophilic infections; 2) the increased spread of *T. rubrum* seems to be related to urban areas with dense populations and social activities, like travelling and sports; 3) the increase of migration leads to the reintroduction of other antropophilic species.

Treatment of dermatophytosis

Usually, the treatment of dermatophytosis begins with topical antifungals. These treatments are used in non-extensive lesions and can´t be irritant and must be well tolerated.[44] However, there are some cases of *tinea* that are not responsive to topical treatments (e.g. some cases of *tinea unguium, tinea capitis, tinea barbae,* widespread skin lesions and skin lesions with folliculitis). In these cases, systemic antifungal therapy is necessary.[44] The majority of antifungals are fungistatic.[44] They should act in the fungus and have low or no activity on the host cell.[45] In some cases of dermatophytosis, bacterial infections may be present (superinfection). Because of this, an antimicrobial with both antibacterial and antifungal action may be useful.[46]

The control of dermatophytic infections requires prolonged therapy, and the existing drugs, on long run seem to exhibit side effects, which requires new biocompatible formulations for long term therapies.[47]

Conventional therapies

The first oral agent used to treat a dermatophyte infection was griseofulvin. Professor Jimmy Gentles discovered this antifungal agent in 1958. He discovered that an antibiotic agent produced by *Penicillium griseofulvum* had an antidermatophytic activity. Griseofulvin is still used to treat dermatophytosis along with azole antifungals and terbinafine introduced many years later.[36]

There are several antifungal therapies to treat *tinea*. Table 4 shows a list of the approved FDA treatments for some types of dermatophytosis. The primary treatment indicated for *tinea corporis*, *tinea cruris*, *tinea pedis* and *tinea manuum* consists in topical medications. There are many topical agents that are available in cream, gel, lotion and shampoo formulas. The agents from azole antifungal family (e.g. clotrimazole, miconazole, econazole, oxiconazole) are the most used. Agents from the allylamine family, as terbinafine and naftifine, are also used.[46]

Oral therapies are the primary treatments in the case of *tinea unguim* (onychomycosis) and *tinea capitis*. However, there is some evidence that suggests that in less severe cases of onychomycosis, a topical treatment, with ciclopirox and amorolfine, can be effective.[46] The most common topical preparations used for *tinea pedis* and *tinea manuum* are formulations of terbinefine, butenafine, miconazole, econazole, ketoconazole, clotrimazole, oxiconazole and ciclopirox. If the infection is chronic, oral antifungals may be used.

Terbinefine, butenafine, econazole, miconazole, ketoconazole, cloritromazole and ciclopirox are some of the topical therapies for treatment of *tinea corporis* and *tinea cruris*. However, topical therapies can eradicate only small, infected areas. So, to treat larger areas or when the infection is chronic or recurrent, oral therapy may be needed. To treat *tinea capitis*, oral therapy is required because it can penetrate the hair shaft while topical therapies are unable to access this deep. However, topical antifungals can be used to prevent reinfections or treat asymptomatic carriers. *Tinea unguium* is difficult to cure. Finger nails presents higher treatment success that toe nails, because they grow faster and the doses of antifungal used are smaller. In subjects with *tinea unguim*, nail debridement

may be a complement to antifungal therapy. Sometimes is necessary to remove the nail plate surgically to eliminate most of the living fungi.[46]

There are several studies on the antifungal action of these agents upon dermatophytes. In table 5, a list of minimum inhibitory concentrations (MICs) of five antifungal agents commonly used upon some dermatophytes is presented.

Mechanisms of action: The mechanisms of these antifungal agents are diverse. Table 6 shows the relationship between some antifungal agents and their correspondent mechanism of action. Although these antifungal agents are currently used to treat ringworm infections, some studies show that there are cases of dermatophyte resistance. For example, Mukherjee et al.[51] (2003) described the existence of a *T. rubrum* strain resistant to terbinafine and Ghelardi et al.[52] (2014) pointed the existence of several *T. rubrum* strains resistant to itraconazole.

Resistance: Resistance can be intrinsic or acquired. In intrinsic resistance, a specific characteristic responsible for resistance is inherent to the species and appears in the process of evolution. This type of resistance enables all members of a species to tolerate an antifungal drug. Acquired resistance happens when a resistant strain appears in a population that was drug-sensitive previously.[45]

Antifungal misuse is the main cause for the emergence of drug resistant strains. If patients do not finish the full course of the treatment or if they make an inadequate use of the antifungal drugs, this could lead to the failure of eliminating the fungus completely, encouraging the growth of more resistant strains.[45]

The mechanisms present in these fungi that contribute to resistance are: modification of target enzymes by mutation, increased drug efflux, stress adaption and over-expression of target enzymes. From a molecular point of view, these biochemical changes can come from gene amplification, gene

transfer, gene deletion, point mutations, loss of *cis*-acting regulatory elements, loss or dysfunction of trans-acting factors, transcriptional activation, hypo or hyper-methylation and stress induced production of alarmones.[53]

Table 6 shows the relationship between the antifungal agent, the corresponding mechanism of action and the common resistance mechanisms for these drugs. Notice that the list of resistance mechanisms presented on that table concerns the dermatophyte *T. rubrum,* because there are few studies regarding drug resistance mechanisms in other dermatophytes and most of the studies pertain to this particular fungus.[45] Due to an increasing resistance of these conventional antifungals, treatment failure is quite recurrent, [54] and, as a result new, alternative, natural antifungal substances, began to be studied.

Essential oils/plant extracts

Humans have used medicinal plants as remedies for centuries. These plants are part of traditional and modern medicine. In some countries of Africa, Asia and Latin America, traditional medicine is still used in primary health care needs.[55] Essential oils are natural complex compounds composed of terpenes, aromatics and terpenoid molecules.[54,56] They possess a strong odor and are liquid, volatile, limpid, rarely colored and soluble in lipids and organic solvents. Usually, they are less dense than water.[56] These metabolites can be synthesized by all plant organs (buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood and bark) and they are stocked in different structures, like secretory cells, cavities, canals, epidermic cells and glandular trichomes.[56] In nature, essential oils have a protective function. They act as antibacterials, antifungals and insecticides for the plants and shield plants against herbivores by reducing their appetite for the plants. These compounds may also attract some insects, which favor the dispersion of pollens and seeds and repel the undesirable ones.[56]

In order to obtain essential oils, the plant material must be distilled using water steam or by

dry distillation. To extract compounds from *Citrus* spp. fruits, the process employed must be cold pressing (also called expression). The plants used for essential oils production could be wild or cultivated.[54]

Essential oils present bactericidal, virucidal, fungicidal, anti-inflammatory, spasmolytic, analgesic and sedative properties and have associated different and characteristic fragrances. Because of that, they are used in cosmetics and medicine.[54,56] In table 7, the main essential oils produced worldwide are listed. Many authors studied the antifungal action of various plants upon dermatophytes. In table 8, a list of minimum inhibitory concentrations (MICs) of some common plants extracts upon two dermatophytes, *T. rubrum* and *M. canis* is presented.

Mechanisms of action of essential oils: Essential oils have been screened for their antifungal activity but the interaction between these oils and microorganisms is still poorly understood. Regarding the number of studies on this topic, there is very little information about antidermatophitic activity of essential oils, since *Candida* spp. and *Aspergillus* spp. have been the most studied species.[54]

Several mechanisms of action of these compounds have been studied. Some are: 1) the reduction of ergosterol content by impairment of its biosynthesis (as suggested by Pinto et al.[63] (2006) that showed that 0.08 μL/mL of *Thymus pulegioides* oil was able to reduce around 70% of ergosterol content of *T. rubrum*); 2) damage of the cell membrane and cell wall and lysis of the mycelia (as indicated by Inouye et al.[64] (2006) that pointed through scanning electron microscopic observations that oregano essential oils were able to damage the cell membrane and cell wall in a dose and time dependent manner); 3) destruction of the inner mitochondrial membranes and cell wall as well as expansion of endoplasmic reticulum near cell membranes (as observed by Park et al.[65] (2007)) by transmission electron microscopy observation of *T. mentagrophytes* hyphae treated with eugenol, a main compound in *Syzygium aromaticum* essential oil); 4) adverse effects on spore germination (a

study of Bajpai et al.[66] (2009)) showed that *Nandina domestica* oil affected spore germination of various strains of *T. rubrum, M. canis* and *T. mentagrophytes*).

In general, the compounds of essential oils that contribute most for their antifungal action are mainly phenolic terpenes such as, carvacrol and thymol. These phenolic compounds can attack cell walls and membranes, affecting the permeability and release of intracellular constituents. So, they might have several invasive targets, allowing a complete inhibition of fungal infection.[66]

Overall, it seems that the antifungal activity of essential oils results from the effect of different compounds on several cell targets and it is not due to a single mechanism of action. Because of that, the occurrence of resistances seems unlikely, since it would be necessary the simultaneous occurrence of several mutations to overcome all the distinct antifungal actions of these compounds.[54]

Chitosan

Chitosan is a cationic polysaccharide composed of β-1,4 linked D-glucosamine and N-acetyl-D- glucosamine residues.[67] It only occurs naturally in some fungi (*Mucoraceae*). On the other hand, chitin is the most abundant polymer in nature after cellulose.[68] It is found predominantly in the shells of crustaceans, the cuticles of insects and the cell walls of fungi.[69] Chitosan can be prepared by cleavage of N-acetyl groups in chitin N-acetyl-2 amino-2-deoxy-D-glucose residues. It's preparation from chitin implies some steps. First, chitin is extracted by acid treatment to dissolve calcium carbonate followed by alkaline extraction to dissolve proteins. Then, a depigmentation step is carried out for removing the astaxantine, allowing a colorless product. After that, a severe alkaline hydrolysis is carried out in order to hydrolyze the acetamine groups of chitin and obtain chitosan.[68] Usually, chitin is isolated from marine crustaceans, because there is a large amount of waste resultant as a by-product of food processing.

Chitosan has proved to be non- toxic, biodegradable, biocompatible, and to possess antimicrobial and anti-inflammatory properties[68], relevant to fight infections caused by

dermatophytes.[70]

Mechanisms of action of chitosan: It is believed that chitosan's cationic nature and high molecular weight helps in its antifungal action, since it interferes with negatively charged residues of macromolecules on the fungal cell surface, thus causing changes in cell membrane permeability.[71,72] It can also prevent DNA transcription to RNA[73] and inhibit the RNA and protein synthesis by permeation into the cell nucleus.[68]

Some authors[73] mentioned that antifungal activity of chitosan is molecular weight dependent and that the smaller the molecular weight is, the stronger the antifungal activity. However, this relationship may not be as linear as some authors suggest, because antifungal activity is dependent on the fungi type; a fungus can interfere with antifungal activity of a drug due to its adaptation and defense mechanisms to stress, which can affect the structural integrity of the cell wall or induce the synthesis of defense compounds.[74] Other works refer that the inhibitory action of chitosan is directly proportional to the concentration, because at higher concentrations of chitosan, fungi will produce higher concentrations of chitinase and this leads to the degradation of chitin and chitosan of fungal cell walls.[73] Additionally to the direct inhibitory effect of chitosan on fungi growth, chitosan also possesses the ability to prevent spore germination. In fact, chitosan, due to its chelating properties, can interfere with the uptake of minerals, particularly Ca^{2+} and nutrients, thereby delaying spore germination.[75] It can, also interact with anionic groups on cell surfaces, due to its polycationic nature, thus causing the formation of an impermeable layer around the cell, which prevents the transport of essential solutes. This polymer can also interact with and flocculate proteins.[68]

For treatment of dermatophytosis, the most relevant chitosan features are its antimicrobial and anti-inflammatory actions. These properties vary with the degree of N-acetylation and the molecular weight of the chitosan molecule. So, both antimicrobial and anti-inflammatory activities are inversely proportional to the degree of N-acetylation. With regard to molecular weight, antimicrobial action

426 can be either directly or inversely proportional to molecular weight, whereas anti-inflammatory action
427 is inversely proportional to molecular weight.[68,69] MIC values for the antifungal activity of chitosan
428 have been reported by several authors ranging from 1.1 mg/mL to 2.2 mg/mL. Balicka-Ramisz et al.[76]
429 (2005) obtained a MIC value of 1.1 mg/mL for *M. canis*. Goy et al.[77] (2009) also determined the MIC
430 values for *M. canis* and the result was 1100 ppm (i.e. 1.1 mg/mL). This is the same MIC value
431 described by Balicka-Ramisz et al. (2005).[76] Both authors also determined the MIC of chitosan for
432 another dermatophyte fungus, *T. mentagrophytes,* and reached the value of 2.2 mg/mL.
433
434 **Conclusion**
435 Dermatophytes are a group of fungi that can invade keratinized tissues of humans and other
436 animals and produce an infection called dermatophytosis. Dermatophytic infections are an important
437 public health problem and to control them a prolonged therapy is required. There are some drugs that
438 can eradicate the infection but, these drugs seem to exhibit side effects and the frequent and prolonged
439 use of these compounds is responsible for strain resistance, representing a potential risk for the
440 environment and human health.
441 In view of all this, new biocompatible drug formulations for long-term use are required. Some
442 natural solutions such as chitosan and plant extracts or essential oils arise as interesting alternatives
443 to conventional treatments. However, the study of their antimycotic properties is in its early stages
444 and more studies need to be done until they can be a reliable treatment option.
445
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Conflicts of Interest

None.

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