

## A STUDY ON ROLE OF KERATINOPHILIC FUNGI IN NATURE: A REVIEW

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### ABSTRACT

The chapter reviews available data on the ability of keratinophilic fungi and include a variety of filamentous fungi mainly comprising Hyphomycetes and several other taxonomic groups. Hyphomycetes include dermatophytes and a great variety of dermatophytes filamentous fungi. Most of the latter occur as saprophytes in soil, and some are plant pathogens. *Chrysosporium* species are the commonest dermatophytes filamentous fungi and are predominantly recovered from soil and other natural substrata by hair baiting technique *C. tropicum* and *C. pannicola* have been frequently isolated from human and animal's skin lesions but their etiological relationship has not been established. The criteria considered important for evaluating the role of dermatophytes filamentous fungi in skin infection are the demonstration of mycelial elements in direct microscopy of skin scrapings or biopsy compatible with those in culture repeated positive cultures from clinical material. The Oils plant has been reported to have antibacterial, antifungal, antiviral, antiparasitic and antidermatophytic properties. It is now considered as a valuable source of natural products for development of medicines against various diseases and also for development of Industrial products. In this review is a compilation of updated information on plant essential oils with antifungal properties.

**Key Words:** Keratinophilic fungi, dermatophytic, antidermatophytic, Human infection, plant essential oils.

### INTRODUCTION

**K**eratinophilic fungi are ecologically an important group of fungi which could be found in soil (Deshmukh, et al., 2006). Some groups of these fungi are causative agents of cutaneous fungal infections named dermatophytosis and the other saprophyte fungi mainly represent hyalohyphomycosis (Palsson, et al., 1968). The prevalence of these fungi depends on different factors such as the presence of nine in the soil sample pH and geographical location (Garg, et al., 1985). Some of these fungi such as

dermatophytes are well known to cause tinea infections which could be transmitted from soil to humans. In general, soil could be considered as a reservoir for human infection. Forests, farmyards, park soils, and sediments of the rivers and oceans containing humus and organic materials are the best candidates for growth of keratinolytic and saprophytic fungi (Ali, et al., 2000). The organisms are transmitted by either direct contact with infected host (humans or animals) or by direct or indirect contact with infected exfoliated skin or hair in combs, hair brushes, clothing, furniture, theatre seats, caps,

bed linens, towels, hotel rugs, and locker room floors. Depending on the species the organism may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a pre-existing injury to the skin such as scars, burns, marching, excessive temperature and humidity. Depending on their habitat, dermatophytes are described as Anthropophilic humans, Zoophilic animals or Geophilic soils. Anthropophilic dermatophytes are the most common sources of Tinea infections (Fardos *et al.*, 2009; Jessup, *et al.*, 2000; Arvind, *et al.*, 2005).

*Trichophyton rubrum* and *Trichophyton tonsurans* are two common dermatophytes. *T. rubrum* found in face, trunk, beard area, nails, feet and groin area infection. *T. tonsurans* found in and thermic and black dot infection. These two species are usually transmitted from person to person. Another common dermatophyte is *Microsporum canis*, which is transmitted from animals such as cats and dogs to humans (Gartner, *et al.*, 1997; Valia, *et al.*, 1994; Washington, 2006; Milne, *et al.*, 2008). Dermatophytes like to live on moist areas of the skin, such as places where there are skin folds. The dermatophyte infection that affects the scalp and hair is known as tineacapitis. It is especially common among school-aged children. For reasons that are not well understood, tineacapitis does not usually occur after puberty. In the recent times few cases of subcutaneous and deep fungal infections have been reported to be caused by dermatophytes. It has been noted that dermatophyte infections are more common in adolescents and adults (Cheesbrough, *et al.*, 2005).

## KERATINOPHILIC FUNGI AND THEIR ROLE IN HUMAN INFECTION

Keratinophilic fungi are a group of fungi that colonize various keratinous substrates and degrade them to components of low molecular weight. These include a host of filamentous fungi representing mainly hyalohypho mycetes and several other taxonomic groups, and some species of yeast-like fungi. Most of the keratinophilic fungi, viz. species of

*Chrysosporium*, *Fusarium*, *Aspergillus*, *Scopulariopsis*, *Curvularia* and *Alternaria* etc. are common saprophytes in soil and plant debris; some of them are often recovered as laboratory contaminants. Many keratinophilic fungi frequently parasitize keratinous tissue, viz. skin, nails and hair in man and animals; some of them share certain morphological features, constituting a special group called dermatophytes. The dermatophytes comprise three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*. Most species of dermatophytes are anthropophilic or zoophilic in their natural habitat, while some occur in soil as saprophytes and are termed geophilic dermatophytes eg. *Microsporum gypseum* and *Trichophyton terrestre* (Rippon, *et al.*, 1988; Kown-Chung, *et al.*, 1992). Some species of keratinophilic fungi, like (Syn. *Hendersonula toruloidea*) and *Phoma* species, are primarily plant pathogens. Over the last three decades, an increasing number of nondermatophytic filamentous fungi have been recognized as agents of skin infections in humans and animals producing lesions clinically similar to those caused by dermatophytes (Punithalingam, *et al.*, 1971). Several investigators have demonstrated that, like dermatophytes, nondermatophytic filamentous fungi can also degrade keratin in vitro and produce proteolytic enzymes including keratinase (English, *et al.*, 1976; Nigam, *et al.*, 1992). This paper attempts to review concisely the present state of knowledge of the biology and ecology of different taxonomic groups of non dermatophytic filamentous keratinophilic fungi and their role in human infection.

The yeast-like keratinophilic fungi are not included in the review. Antifungal susceptibility testing is performed to provide information to allow clinicians to select appropriate antifungal agents useful for treating a particular fungal infection (Isham, *et al.*, 2000). For a definitive therapy also it is essential to evaluate the resistant dermatophytes using a standardized, simple and reproducible in vitro assay to determine the antifungal activity of drugs against isolates. In vitro antifungal susceptibility tests are now mainly used for epidemiological surveys, determination of the degree of

antifungal activity, and the prediction of clinical outcome based upon an optimization of antifungal therapy (Rezaei, *et al.*, 2009). A few antifungal agents are available and licensed for use in veterinary practice or human being treatment. The use of systemic drugs is limited to treat man or animal due to their high toxicity and problems of residues in products intended for human consumption.

Different treatments have been recommended to control dermatophytes several methods have been developed for testing antifungal against this group of pathogens. Multicentre studies to develop a standardized antifungal susceptibility assay were initiated by the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards", NCCLS) in 1983. Dilution tests are widely used in macro- and micro-assays, but these methods are difficult to be used in most laboratories. Recently, studies were done to establish a simple method to solve this problem. The agar-based disk diffusion (DD) susceptibility method for dermatophytes is simple, inexpensive, and does not require specialized equipment. The disk diffusion method has a good correlation with the reference dilution assay. In this study is to determine in vitro activity of four antifungal drugs that are most commonly used to treat dermatophytosis; Miconazole (MIZ), Clotrimazole (CTZ), Fluconazole (FLZ) and Ketoconazole (KTZ). The study is being undertaken to evaluate the optimum method for rapid isolation of dermatophytes and to study its resistance to antifungal drugs (Bahaedinie, *et al.*, 2009).

## ECOLOGICALLY IMPORATANT IN KERATIN DEGRADATION

Keratinophilic fungi are ecologically important group as they play a significant role in the natural degradation of keratin substrate and residues. Occur with the variable distribution patterns, these are dependent on factors like human and animal presence. This group of microorganisms utilize keratin present in the soil Geophilic Species, the certified substrata in humans Anthropophilic Species and animals

Zoophilic Species. This is thus considered to be a natural evolution in keratin utilization and is responsible for organism's pathogenicity Keratinolytic fungi specialize in the decomposition of keratin, being the main component of keratinous substrata. Keratinophilic fungi associate keratinolytic fungi, utilizing non-protein components of the substrata or the products of keratin decomposition (Majchrowicz, *et al.*, 1969). Higher incidence of mycotic infection especially superficial mycoses, cutaneous mycoses and opportunistic mycoses has enhanced the use of various antifungal agents for faster relief. The work in vitro antifungal activity of different synthetic, herbal shampoos and natural products used in traditional medicine was performed on clinical isolates of fungi like *Malassezia sps*, *Trichophyton sps* and *Aspergillus sps*. When compared the effects of test compounds on seven different organisms used, synthetic shampoos showed excellent inhibitory activity on all of them. Of the fungi used *Trichophyton* was most inhibited by all test compounds and shown to be highly sensitive to them.

*Malassezia* is moderately inhibited with two species more sensitive than the other. *Aspergillus* is least inhibited. Though commercial synthetic and herbal shampoos used were potent inhibitors of all the fungi tested natural and herbal preparations were equally good either when used alone or in specific combinations which support their use in traditional medicine (Abdul, *et al.*, 2013). In order to determine the incidence of dermatophytes and related keratinophilic fungi present in Riyadh soil, eighty soil samples were collected. These samples were screened using hair baiting techniques for isolation. Out of a total 80 samples, 69 (86%) were positive for growth of keratinophilic fungi. Eleven genera and 19 species were isolated and identified, of which *Chrysosporium indicum* (33.75%) the most predominant species was isolated followed by *C.tropicum* (26.25%), *Aspergillus flavus* (17.50%), *Microsporum gypseum* (13.75%) and *Trichophyton terrestre* (11.25%). Garden soils, followed by playground soils were found to be the most suitable for fungal growth. Some of the

other fungi isolated were *C. zonatum*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium moniliforme*, *F. solani*, *Aphanoascus fulvescens* etc. To our knowledge, this appears to be the first report concerning the isolation of keratinolytic fungi *M. canis*, and *Trichophyton terrestre*, from soils of Riyadh by hair baiting technique (Rhizwana, *et al.*, 2012). In this study was to isolate and identify keratinophilic a fungus from soils stressed by occurrence of animals, both pets and farm animals. Keratinophilic fungi are present in the environment with variable distribution patterns that depend on different factors, such as humans, and or animals, presence, which are of fundamental importance.

This article draws the attention towards the incidence of fungal opportunistic pathogen (*Pseudallescheri boydii*) in soil sample stressed by occurrence dog. This substrate should be considered as a potential source of the opportunists (*Trichophyton ajelloi*). was representative and encountered in all the samples investigated. A human pathogen, a geophilic dermatophyte *Microsporium gypseum* was isolated from all 6 soil samples, stressed by occurrence of a dog. Another soil samples genus *Chrysosporium* has also occurred, namely *Chrysosporium keratinophilic* and *Chrysosporium queensl* and *icum*. Preliminary results showed that keratinophilic fungi were richly represented in the soils in Slovakia and should pay attention to their occurrence especially in the human environment (Dana, *et al.*, 2013).

## MICROORGANISMS INVOLVED

The content of pathogenic organisms, including fungi, sewage sludge land application poses risks to both human health and the environment. One of the ways for reducing pathogens in sludge is liming. The study to determine the effect of sludge liming on the composition of keratinolytic and keratinophilic fungi in model experiments. The fungi were examined using the hair baiting method and the dilution method with incubation on a Wiegand medium supplemented with chloramphenicol (100 mg/L) and actidione

(500 mg/L). The sludge liming considerably decreased the number of actidione-resistant fungi prop gulesand eliminated many fungal species, including *Pseudallescheri boydii*. The influence of this process on hair-baited fungi was that the liming eliminated keratinolytic and keratinophilic fungi at 37°C. In the range of 23-33°C, the liming considerably restricted the growth of keratinolytic fungi, including *Microsporium gypseum*, but only slightly affected keratinophilic fungi, including *Pseudallescheri boydii*. The sludge liming decreases the risk posed by geophilic dermatophytes and other keratinolytic fungi, as well as by keratinophilic fungi to humans and the environment. The process affected more keratinolytic fungi than keratinophilic (Ulfig, 2005). The frequency and species diversity of keratinophilic fungi in 38 nests of nine species of wetland birds were examined.

Nine species of geophilic dermatophytes and 13 *Chrysosporium* species were recorded. *C. Keratinophilice*, which together with its teleomorph (*Aphanoascus fulvescens*) represented 53% of the keratinolytic mycobiota of the nests, was the most frequently observed species. *Chrysosporium tropicum*, *Trichophyton terrestre* and *Microsporium gypseum* populations were less widespread. The distribution of individual populations was not uniform and depended on physical and chemical properties of the nests humidity, pH (Kowalska, *et al.*, 2011). Soil is a well-known source of wide variety of microorganisms. This article briefly explains the isolation and identification of ecologically important keratinophilic fungi which are involved in the degradation of most abundant and highly stable animal protein – keratin. Soil is the home of several such fungi which are not even noticed. During the course of study approximately 15 different fungal species were isolated and identified. The isolation technique adopted was hair baiting (Vanbreuseghem, 1952). This hurble attempt will create interest among the students to know more about the fungal diversity of soil and their enormous potentials. *Trychophyton*, *Microsporium*, *Chrysosporium*, *Aspergillus*, etc. are some among the isolates (Mathew, *et al.*, 2012).

## BIODEGRADATION OF KERATIN

Facultative Thermophilic bacterial strain capable of producing thermo stable keratinase was isolated from habitats that are naturally thermophilic. Based upon biochemical characterization the bacterium was identified as *Bacillus licheniformis*. Bacteria produced keratinase in the medium containing horn meal as sole source of carbon and nitrogen. Effect of different parameters on enzyme activity was studied. Keratinase was active in broad temperature range from 50°C to 80°C. Optimum temperature was found to be 50°C. Incubation at 70°C for one hour retained 53.84 % residual activity indicating its thermo stable nature. Enzyme also exhibited stability at broad pH range being active from pH 4 to 9. Optimum pH was found to be 7. EDTA at 1mM concentration inhibited the keratinase activity by 50 % while PMSF inhibited the total activity of the enzyme. The study offers interesting potential of enzyme use in hydrolysis of keratinolytic waste for generation of value added products for varied biotechnological applications (Gawade, *et al.*, 2013). The isolate and identify a new local bacterial strain, which is able to completely degrade keratin rich wastes into soluble and useful materials which can be used for many purposes. Bacterial keratinases are of particular interest because of their action on insoluble keratin substrates and generally on a broad range of protein substrates (Ton Sinoy, *et al.*, 2011).

## ROLE OF MICROBIAL ENZYMES

These enzymes have been studied for de-hairing processes in the leather industry and hydrolysis of feather and keratin. Samples from poultry wastes, soil, water, and feather were collected from different places in Parbhani. Each sample was placed on feather meal agar plates containing 5 g LG1 feather as the sole carbon and nitrogen source and the obtained colonies were selected, purified and their growth were detected on casein agar medium. The well grown isolates on casein agar medium which producing the largest clearing zone on casein plate were selected for keratinase assays. Out of 16 bacterial isolates, 5 isolates were selected. The

best keratinase producing bacterium kea MS21 was selected and identified based on morphological, physiological and some biochemical characteristics. It was recorded as a species belonging to the genus *Pseudomonas* and identified as *Pseudomonas* sp. Precipitation and purification of the keratinase enzyme in addition to factors affecting enzyme activity (pH & temp.) were studied. The enzyme molecular weight was determined to be of 30 KD using sodium dodecyl sulphate polyacrylamide gel electrophoresis analyses. The optimum temperature and pH were determined to be 35°C and pH 8.0, respectively. The effect of some protease inhibitors and activators were also studied (Bhausaneb, *et al.*, 2011). Dermatophytes are a group of closely related fungi that have the capacity of invading the keratinized tissue (skin, hairs and nails) of human and other animals to produce infections known as dermatophytoses, which are commonly referred to as ringworm (Gugnani, 2000). Dermatophytes can digest keratin and other proteinaceous substrates present in skin and its appendages, such as nail, hair, and feather, and use it as its sole source of carbon and nitrogen.

Proteolytic and keratinolytic activities of dermatophytes have been a subject of interest for several years to understand the pathogenicity of infection (Venkatesan, 2010). These dermatophytes are also called keratinophilic fungi because of their high affinity for keratin. Keratin is a refractory protein polymer only produced by man and animals; it is the main constituent of epidermal skins, hairs, feathers, reptilian scales, quills, horns, hooves and nails. When these materials are shed into the soil and other potentially moist substrata such as disused nest, they are principally degraded by keratinophilic fungi (Summerbell, *et al.*, 2000). The process of degradation of keratin contained in its natural forms such as hair or feathers seem to be a result of both the mechanical action of the fungus and the activity of enzymes. The invasion of hair by anthropophilic dermatophytes has been investigated with light and electron microscopy under natural and experimental conditions. Studies by scanning

electron microscopy of scalp hair from subjects infected with *Trichophyton violaceum* (Tosti, *et al.*, 1970). Guinea pig skin infected experimentally with *T. mentagrophytes* (Heath, *et al.*, 1970) showed the hypha growth during in vivo infection. These morphological observations indicate that dermatophytes grow in filamentous form during in vivo infection of hair following the same pattern as on or dinary laboratory media. Since mycelium and the remaining insoluble substrate cannot be separated, quantitative measurement of keratin degradation poses a problem and hence only the loss of total dry weight (keratin + mycelium) were calculated (Ziegler, *et al.*, 1963). Calculated the net loss of weight of the substrate by evaluating the 'economic coefficient'. It is believed that the rate and completeness of the keratin degradation is, dependent on the kind of substrate and correspond roughly to its hardness, viz., cystinecontent (Kunert, *et al.*, 2000).

Two major reasons to examine keratinolytic fungi in the environment can be named. First, the abundance of these microorganisms is observed in environments rich in keratinous remnants of human and animal origin and in other substrata needed for fungal growth, e.g., in soils of highly populated and animal-inhabited areas, sewage sludge and municipal waste (Deshmukh, *et al.*, 1998). Keratinolytic fungi play the main role in the decomposition of these substrata and can be used for biotechnological applications (Onifade, *et al.*, 1998). Second, keratinolytic fungi display potential pathogenic properties to animals, including humans (Filipello-Marchisio, *et al.*, 2000). Therefore studies of these fungi in the environment are of epidemiological importance. Keratinolytic fungi also occur in abundance in highly industrialized and polluted areas, in which organic and inorganic contaminants considerably affect microbial populations. Therefore, an essential problem is an evaluation of the effects of these contaminants, including oil hydrocarbons on fungal distribution in these areas. The role of keratinolytic fungi in biodegradation of these hydrocarbons is also to be explained. Extensive studies have recently been carried out to explain the factors influencing keratinolytic fungi in the

environment (Jamous, *et al.*, 2000). An attempt was made for the second time in India to study the occurrence of keratinophilic fungi in museums objects. Samples collected from fur and leather, their surroundings and deposited dusts from five museums (one control) were baited for the isolation of keratinophilic fungi. During the study a total of four keratinophilic fungi (*Chrysosporium keratinophilum*, *C. tropicum*, *C. indicum* and *Microsporum gypseum*) and four non-keratinophilic fungi (*Aspergillus niger*, *A. flavus*, *A. fumigates* and *Fusariumoxy sporum*) were isolated. Among the isolated keratinophilic fungi, *Chrysosporium keratinophilum* (RIV=134.18) and among the non-keratinophilic fungi, *Aspergillus niger* (RIV=140.93) were found to be dominant. And among all the five museums designated from M1 to M5, the M1 was Visakha Museum, Visakhapatnam, India, which has shown least possible prevalence of the mycoflora, was taken as control. Among the remaining four museums, M3 has shown the highest growth and M2 has shown the lowest growth of the mycoflora (Maruthi, *et al.*, 2013).

## EPIDEMIOLOGY OF NAIL INFECTION OF KERATINOPHILIC FUNGI

The epidemiology of fungal infections of the nails, or onychomycosis, is only partially known since it is a combination of different factors which include, among others, the etiological agent, the clinical form of the infection, the patient's background, other parameters related to the physiology and ecology of the causative fungi, and even the habits of the population. This chapter is a review of the data available on the most frequent etiological agents as well as several occasional ones, and includes the methods used for a more accurate diagnosis. Although there is no doubting the importance of dermatophyte fungi, especially *Trichophyton rubrum*, as the causative agents of tined unguis, the etiological role of some keratinophilic yeasts and moulds is more controversial as they are so-called opportunistic agents of onychomycosis. To a large extent the clinical forms of onychomycosis refer to a particular fungus or group of fungi, some of which like *Scytalidium*

/Hendersonula for example, are found in certain regions with a warm climate. Although there is the belief that onychomycosis has increased its presence especially in developed countries, the data available on its prevalence is limited and varies considerably according to the origin of the publication and the study method. Consideration is given to the factors favouring infection, the low incidence of this mycosis among the younger age group, and the social and economic impact of onychomycosis in developed countries (josep, *et al.*, 2000).

Alkaline proteases are of considerable interest in view of their activity and stability at alkaline pH this review describes the proteases that can resist extreme alkaline environments produced by a wide range of alkalophilic microorganisms. Different isolation methods are discussed which enable the screening and selection of promising organisms for industrial production. Further, strain improvement using mutagenesis and recombinant DNA technology can be applied to augment the efficiency of the producer strain to a commercial status. The various nutritional and environmental parameters affecting the production of alkaline proteases are delineated. The purification and properties of these proteases is discussed and the use of alkaline proteases in diverse industrial applications. (Ganesh, *et al.*, 1999). The effect of licid lotion (recommended by professionals for killing and protection against head lice and nits) on the presence of fungi on 96 human hair samples were studied by plating the untreated and licid-treated hair directly on Sabouraud's dextrose agar medium and by using soil plating technique. Licid caused an inhibition in frequency of occurrence of all fungal genera recovered on treated hair samples using soil plating technique, when compared with those of un-treated specimens. However, the frequency of occurrence of each of *Aspergillus*, *Penicillium* and *Rhizopus* was promoted by licid using direct plating technique. The capabilities of selected four fungal species to degrade keratin in licid-treated liquid medium were also tested. The quantities of amino-N and protein-N in culture media (containing 2.6% human hair as a keratinase substrate) inoculated with each of

*Alternation alternate*, *Chrysosporium indicium*, *C. keratinophilum* and *C. tropicum* and supplemented with different concentrations of licid (500, 1000 and 1500  $\mu\text{g ml}^{-1}$ ) were increased (Abdel, *et al.*, 1995). A culture medium was optimized for the production of extra cellular keratinase by a newly isolated strain of *Bacillus sp.* from Goa, India in shake-flask culture. The keratinase production was increased by approximately 40-fold when the strain was grown in an inexpensive optimized medium ( $120 \text{ U.mL}^{-1}$ ) compared to the un-optimized medium ( $3.80 \text{ U.mL}^{-1}$ ). The maximum amount of keratinase activity was produced at  $37^\circ\text{C}$  when the bacterium was cultured for 72 h in medium containing feather meal as the sole source of carbon and 0.025% yeast extract with initial pH of 7.0 under submerged fermentation. The isolated keratinolytic *Bacillus sp* also exhibited remarkable feather degrading ability. These results suggest potential biotechnological applications of this bacterium that involve hydrolysis of keratin, including the improvement of the nutritional properties of feathers (and other keratins) used as supplementary feedstuffs (Kanchana, 2012). Poultry farm soil samples collected from different localities of Ernakulum and Thirstsur districts of Kerala were screened for the keratinolytic fungi. During the course of study 8 different fungi were isolated and identified. *Aspergillus*, *Chrysosporium*, *Microsporium*, *Trychophyton* and *Penicillium* were the fungi isolated and were grown in wheat bran substrate. Feather keratin powder was added to the substrate to enhance the enzyme production. They were found utilizing keratin substrate releasing keratinase enzyme into the medium. These enzymes were assayed for their activity. Some cultural conditions were tested to attain maximum keratinase production. Maximum enzyme production was reached on the 4th day of incubation of the culture at  $37^\circ\text{C}$  and pH 8.5 (Mini, *et al.*, 2012-13).

## FACTORS AFFECTING KERATIN DEGRADATION

The spore morphologies are a major character in fungal taxonomy, although many isolates are not able to sporulate on common artificial media.

Environmental factors play an important role in the growth and sporulation of dermatophytic and keratinophilic fungi. Fungi grow best at optimum temperature and related humidity. Both the factors govern metabolic activities of growing organism. The extremely high and very low temperature decreases the growth of keratinophilic fungi. The increased level of relative humidity shows excellent growth. In the present study various temperature regimes i.e. 0°, 5°, 10°, 15°, 20°, 25°, 30°, 35°, 40°, 45°, 50°C and different relative humidity i.e. 11.05%, 22.45%, 33.00%, 50.00%, 62.00%, 75.00%, 95.00% were used to evaluate the growth and sporulation of *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis* and *Mycosporum gypseum* (Chandra, *et al.*, 2012). The variety and galaxy of fungi and their natural beauty occupy prime place in the biological world and India has been the cradle for such fungi. Only a fraction of total fungal wealth has been subjected to scientific scrutiny and mycologists have to unravel the unexplored and hidden wealth. One third of fungal diversity of the globe exists in India. Out of 1.5 million of fungi, only 50% are characterized until now. Unfortunately, only around 5–10% of fungi can be cultured artificially. Fungi are not only beautiful but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as bio fertilizers and many other ways. Fungal biotechnology has become an integral part of the humans (Manoharachary, *et al.*, 2005). Higher incidence of mycotic infection especially superficial mycoses, cutaneous mycoses and opportunistic mycoses has enhanced the use of various antifungal agents for faster relief. In the present work, in vitro antifungal activity of different synthetic, herbal shampoos and natural products used in traditional medicine was performed on clinical isolates of fungi like *Malassezia* spp., *Trichophyton* spp. and *Aspergillus* spp. When compared the effects of test compounds on seven different organisms used, synthetic shampoos showed excellent inhibitory activity on all of them. Of the fungi used, *Trichophyton* was most inhibited by all test compounds and shown to be highly sensitive to

them. *Malassezia* is moderately inhibited with two species more sensitive than the other. *Aspergillus* is least inhibited. Though commercial synthetic and herbal shampoos used were potent inhibitors of all the fungi tested, natural and herbal preparations were equally good either when used alone or in specific combinations which support their use in traditional medicine (Prathusha, *et al.*, 2013). Soil sample collected from different localities frequented particularly by various, animals, and birds were baited for the isolation of fungi capable for colonizing and attacking keratinous substrates. In all 94 fungi were isolated distribution of keratinophilic fungi in various types of soil and on different kind of keratin substrates are discussed. Some fungal species new to India fungal flora have been also mentioned (Agrawal, *et al.*, 1976). Keratins are proteins with extremely high molecular weight. They are resistant to digestion by pepsin and trypsin, insoluble in dilute acids, alkalis, water and organic solvents. They have high sulphur content amino acid in the form of cysteine

The resistance to solvents and enzymes is due to close packing of these chains. Fungi capable of colonizing natural keratin such as skin, feathers, hair, horn, hoof etc. are widespread in nature and probably fulfil a vital function in the breakdown of hard keratin detritus of man and animals to simple organic compounds (Ajello, *et al.*, 1953; Gordon, *et al.*, 1953). These fungi are known to have a specialized enzymatic system which enables them to break down the keratin, a complex protein to simple organic compounds. In the study an attempt has been made to study the ability of a keratinophilic fungus, *Microsporum gypseum* to degrade pigeon feathers, peacock feathers and chicken feathers. The addition of era tin substrates stimulated the growth of keratinophilic fungi. They deteriorate them very rapidly and release high amount of protein (Parihar, *et al.*, 1999; Kushwaha *et al.*, 1983). It is now well established that the breakdown of keratin is carried out by the action of extracellular enzymes, keratinases (Biswas, *et al.*, 1988; Malviya, *et al.*, 1992). The ability of the keratinophilic fungus *Microsporum gypseum* to degrade different keratin substrates viz.,



chicken feathers, pigeon feathers and peacock feathers has been studied under different incubation periods. The amount of net protein (7g/ml) released during the growth of *Microsporium gypseum* on different keratin substrate reveals that pigeon feathers are most degraded keratin substrate and peacock feathers are least degraded keratin substrate (Sowjanya, *et al.*, 2012).

## ESSENTIAL OILS FOR TREATMENT OF MYCOSES

The Oils from medicinal plants were screened for their activity against *A. fumigates* and *A. niger* by disc diffusion method. Minimum inhibitory concentrations (MIC) of oils against *Aspergillus fumigates* and *Aspergillus niger* done by agar dilution method and Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentration (MCCs) data (%v/v) obtained by the broth micro dilution method. The maximum antimicrobial activity was demonstrated by oils of *Cymbopogon martini*, *Eucalyptus globules* and *Cinnamomum zylenicumas* compared to control, followed by *Cymbopogon citratus* which showed activity similar to control (miconazole nitrate). The oils of *Menthaspicata*, *Azadirachtaindica*, *Eugenia caryophyllata*, with *aniasomnifera* and *Zingiberof ficinaleex* inhibited moderate activity. The oils of *Cuminumcyminum*, *Allium sativum*, *Ocimum sanctum*, *Trachyspermum copticum*, *Foeniculumvulg* and *Elettaria cardamomum* demonstrated comparatively low activity against *A. niger* and *A. fumigatus* compared to control. Mixed oils showed maximum activity as compared to standard. These results support the plant oils can be used to cure mycotic infections and plant oils may have role as pharmaceutical and preservatives (Bansod, *et al.*, 2008). Various plant materials are believed to have antifungal activity and many essential oils have been reported to have antifungal activities with no side effects on humans and animals (Sokmen, *et al.*, 1999). Previous in vitro and in vivo investigations suggested that the essential oils could be used as effective antifungal agents (Adam, *et al.*, 1998). The selection of plants for evaluation was based on traditional usage for

treatment of infectious diseases (Janssen, *et al.*, 1986; Crespo, *et al.*, 1990) However; there are only limited data available on the antifungal activity of essential oils against human and plant fungal pathogens. Fungal species of the genera *Aspergillus*, *Fusarium* and *Alternaria* have been considered to be major plant pathogens Worldwide (Ghafoor, *et al.*, 1976). The increasing resistance to antifungal compounds and the reduced number of available drugs led us to search for the new alternatives among aromatic plants and their essential oils, used for their antifungal properties. The antifungal activity can be attributed to the presence of some components such as carvacrol,  $\alpha$ -terpinyl acetate, cymene, thymol, pinene, linalool which are already known to exhibit antimicrobial activity (Knobloch, *et al.*, 1985; Cimanga, *et al.*, 2002). Plant essential oils are potential source of antimicrobials of natural origin. Essential oils obtained from many plants have recently gained a great popularity and scientific interest. Consumer demand for natural preservatives has increased, whereas the safety aspect of chemical additives has been questioned. The plant oil has been reported to have antibacterial, antifungal, antiviral, antiparasitic and antidermatophytic properties. It is now considered as a valuable source of natural products for development of medicines against various diseases and also for the development of industrial products. In this review is a compilation of updated information on plant essential oils with antifungal properties (Vidyasaga, *et al.*, 2013). Fungi are a natural part of our environment and play an important role in decomposition of organic matter. They can grow on almost any material if enough moisture is available and cause damage to the structures; decorations are also responsible for the indoor air quality (Verma, *et al.*, 2008). In the antifungal activity of essential oils of selected plant species, viz. *Piper nigrum* Linn., *Ricinuscommunis* Linn., *Cedrusdeodara* Roxb. Loud., *Syzygiumaromaticum* Linn. Merrill & Perry, *Eucalyptus globules* Labill., *Citrusaurantium* Linn., *C. limon* (Linn.) Burm. F. *Oleaeuropaea* Linn & *Menthapiperita* Linn. was assayed for fungi toxicity against two genus, viz. *Aspergillusniger* and *Geotrichum candidum*. The highest and broadest activity was

shown by the essential oils of *S. aromaticum*, *C. limon*, *C. aurantium* and *M. piperita*, while the oils of *R. communis*, *C. deodara* and *O. europaea* demonstrated the lowest level of antifungal activity among the oil tested as compared to standard drug, Ketoconazole. The 5 ppm concentration of essential oils of *S. aromaticum*, *C. Limon* and *M. piperita* completely inhibited the mycelia growth of *A. niger* and *G. Candidum* to the same extent as 5 ppm of Ketoconazole. However, the 5 ppm concentration of essential oil of *C. aurantium* completely inhibited the mycelial growth of *G. candidum* at 10 ppm concentration to the same extent as 5 ppm concentration of Ketoconazole in positive control (Chaurasia, *et al.*, 2011).

### CONCLUSION

It is apparent that there are numerous dermatophytic filamentous keratinophilic fungi belong to diverse taxonomic groups. Hyphomycetes include dermatophytes and a great variety of dermatophytes filamentous fungi the later occur as saprophytes in soil and some are plant pathogens the species are the commonest dermatophytes filamentous fungi and are predominantly recovered from soil and other natural substrata by hair baiting technique. The oils plant has been reported to have antibacterial, antifungal, antiviral, antiparasitic and antidermatophytic properties in this review is compilation of updated information on plant essential oils with antifungal properties. The keratinophilic fungi with potential to cause skin infections in human and animals are likely to be discovered, newly known antimycotics should be tried to treat such infection. Investigation of proteolytic enzymes including keratinase of these fungi is an area where further research is needed. Keratinase enzyme play important role in degradation of various keratin substrate and also help in maintaining environment ecofriendly by minimize soil and water pollution.

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