1.0 INTRODUCTION

Dermatophytes are the most significant fungi because of their widespread involvement of population at large and their global prevalence. They are assuming greater significance due to the use of immunosuppressive drugs and some important disease conditions such as diabetes mellitus etc. Hot and humid climate of the tropical and subtropical countries like India is suitable for these fungi which make dermatophytosis a very common superficial fungal skin condition (Sumathi et al., 2013).

Dermatophytosis is the infection of keratinized tissues such as the epidermis, hair and nails. This condition is caused by a group of closely related filamentous fungi belonging to the genera: *Epidermophyton*, *Microsporum* and *Trichophyton*. They are commonly known as dermatophytes which degrade the keratin with the help of keratinases and invade the superficial skin tissue. The infections due to these pathogens are generally cutaneous and restricted to the non-living, cornified layers of the skin. However, in chronic conditions, the fungi may invade deeper tissues. The concurrent infections with other organisms in particular lead to chronic infections. The dermatophytes, in general, lack the ability to invade deeper tissues or organs of the host. The infections due to them are generally referred to as ‘ringworm’ infections due to ring like appearance of the skin lesions produced by them. These infections are also known as ‘tinea infections’ and are named according to the location of the lesions on the body e.g. tinea capitis refers to ring worm infection of head region and tinea pedis is the infection of the feet. Depending on their habitat, dermatophytes are described as anthropophilic (human), zoophilic (animal) and geophilic (soil). Anthropophilic dermatophytes are the most common sources of tinea infections.

Dermatophytosis may reach epidemic proportions in areas with high rate of humidity, over-population and poor hygienic conditions (Weitzman & Summerbell, 1995; Peerapur et al., 2004). Dermatophyte infections have worldwide occurrence, affecting 20% to 25% of the world's population. The incidence continues to increase progressively (Havlickova et al., 2008). Dermatophytes grow best in warm and humid environments and are, therefore, more common in tropical and subtropical regions. *Microsporum canis*, *M. manum*, *Trichophyton mentagrophyte*, *T. verrucosum* and *T. equinum* are some of the important dermatophytes that are of worldwide occurrence. *T. simii* infects monkeys only and has been reported from Asia.
only, and *T. mentagrophytes* var. *erinacei* infections were reported in France, Great Britain, Italy and New Zealand. *T. rubrum* is the causative agent in most developed countries and is implicated in most cases of tinea unguium, tinea cruris, tinea corporis and tinea pedis (Foster *et al.*, 2004; Borman, 2007). Factors such as change in migration pattern, growth in tourism, and changes in socioeconomic conditions are responsible for variation in the epidemiological pattern of dermatophytes. There are very little epidemiological data in respect of the developing world because only a few studies have been conducted in relation to the etiology of superficial mycoses (Weitzman and Summerbell, 1995). It is difficult to establish the overall incidence and prevalence of various dermatophyte infections in different parts of the world because the studies of one region of the country may not be a true representation of the overall disease pattern of that country (Ameen, 2010).

The clinical presentation, though very typical of ringworm infection, is very often confused with other skin disorders, making confirmatory laboratory diagnosis mandatory (Huda *et al.*, 1995). The introduction of new exotic pets, active participation of players in overseas sporting events and involvement of new strains of dermatophytes that have not been reported earlier for accurate identification (Jun and Kim, 2004; Jung *et al.*, 2008; Lee *et al.*, 2009). The conventional methods of identification of dermatophytes based on clinical manifestations, colony characteristics, the appearance of hyphae and macro and micro-conidia under light microscope and biochemical characterization of the dermatophytes which are routinely used in the microbiology laboratory. These methods are time consuming and require skilled/expert personnel for accurate identification of dermatophyte species. The molecular characterization might help in understanding the epidemiology of dermatophytosis, which is quite important for their control. Molecular studies play a significant role in providing a classic picture of the taxonomy and the current epidemiological outbreaks since the epidemiological profiles may differ from place to place due to frequent change in climatic conditions, environmental and socio-economic status and migration rate etc. (Elavarashi *et al.*, 2014). Several molecular techniques have been used for the diagnosis of dermatophytosis; polymerase chain reaction (PCR) (Malinovschi *et al.*, 2009), nested PCR (Kanbe *et al.*, 2003a), multiplex PCR (Kim *et al.*, 2011), arbitrary primed-PCR (AP- PCR) (Liu *et al.*, 2000), amplification of rRNA gene internal transcribed spacer regions (Jackson *et al.*, 1999), sequencing of 28S ribosomal DNA (Ninet *et al.*, 2003)
etc. These methods provide rapid, accurate and differential identification. Additional analysis of the ITS regions provides a simple and reproducible molecular method for identifying dermatophyte species. This method utilizes PCR amplification of ITS-1 and ITS-2 regions. In the present study, we planned to utilize the primers for amplification of these regions both for strain typing and species identification of the dermatophyte isolates recovered from superficial mycoses of human patients as an adjunct to the conventional methods. These rapid diagnostic methods aid in accurate treatment of dermatophytosis and help to develop effective preventive measures.

During past two decades, the incidence of dermatophytic and other fungal infections has increased considerably. The management of dermatophytosis begins with the application of topical agents which should penetrate the skin and remain there in order to suppress the fungus. A number of antifungal drugs have been introduced for treating cases of dermatophytosis. The choice of treatment is based on factors such as site and extent of the infection, the species involved, efficacy and safety profile and kinetics of the drug. The topical agents are effective in case of localized non-extensive lesions caused by dermatophytes while the oral preparations are required in case of nail infections, and extensive folliculitis (del Palacio et al., 2008). Triazoles (itraconazole, fluconazole), imidazole (ketoconazole), allylamine (terbinafne) and griseofulvin have been reported to have substantial inhibitory activity against dermatophytic conditions (Gupta and Dell-Rosso, 2000). However, regeneration of the infections has been reported with the cessation of therapy (Mukherjee et al., 2003). In vitro antifungal susceptibility testing could help to optimize the therapy and to select an effective antifungal agent (Carrillo-Munoz et al., 2006). The results of susceptibility testing have been found satisfactory by broth macro-dilution and micro-dilution tests by various workers (Fernández-Torres et al., 2000; Jessup et al., 2000; Fernández-Torres et al., 2003). Clinical and Laboratory Standards Institute (CLSI) produced M38-A2 document which is now a standard method for susceptibility testing of filamentous fungi including dermatophytes (CLSI, 2008). We have used this protocol in the present study for determining antifungal susceptibility testing of dermatophyte isolates recovered from human patients against itraconazole, terbinafine and ketoconazole.

The climatic conditions of our country are favourable for maintenance of dermatophyte infections situated within the tropical and subtropical belts of the world, India has remarkably
varied topography which favours fungal growth (Singh and Beena, 2003a). Cases of superficial mycoses in the country were first reported from upper Assam by Dr. Powell in 1900. Since then, the prevalence of dermatophytes has been reported from different states of the country. Most common clinical condition is tinea corporis, followed by tinea cruris, tinea capitis, tinea faciae and tinea unguium. T. rubrum (43.7%) is the most frequent isolate, followed by T. mentagrophyte, 28.1%, Epidermophyton floccosum, 7.8% and Microsporum audouinii, 6.2% (Peerapur et al., 2004). Tinea capitis is common in children between 5-9 years of age and prevalence varies with geographic regions, seasonal conditions, and hygienic and other living conditions. Various studies on the prevalence of dermatophytosis have been conducted in different parts: Chennai (Venkatesan et al., 2007), Madhya Pradesh (Pandey and Pandey 2013), Andhra Pradesh (Madhavi et al., 2011; Maruthi et al., 2012), West Bengal (Grover and Roy, 2003; Das et al., 2009), Gujarat (Singh and Beena, 2003a; Bhavsar et al., 2012), Chandigarh (Chakrabarti et al., 1992), Karnataka (Reddy et al., 2012) and few other states. The distribution, frequency and the causative agents involved vary from place to place depending upon the climatic, socioeconomic conditions and the population density. The state of Himachal Pradesh has ideal conditions for the superficial mycoses. Fourteen species of keratinophilic fungi including dermatophytes were recovered from 130 soil samples from the Ladakh region by Deshmukh et al., 2010. This study suggests that dermatophytes are quite tolerant and have the potential to adapt to various biotic and abiotic factors. The prevalence of onycomycoses among farmers and office workers to the tune of 20% each has been reported from Shimla, another colder region of the state (Gupta et al., 2007). These workers reported the prevalence of T. rubrum, T. mentagrophyte and T. verrucosum in this geographical region. The implication of M. gypseum in tinea corporis in a HIV patient at Indira Gandhi Medical College, Shimla has been reported by Bhagra et al., (2013). This dermatophyte is uncommon in tinea corporis and represents atypical dermatophytosis. The incidence of dermatophytosis has also been reported in bovines of the Kangra valley of Himachal Pradesh (Chahota et al., 2000).

The availability of scanty data on the prevalence and associated epidemiological factors of dermatophytosis in the state of Himachal Pradesh prompted us to take up the present study which utilizes conventional methods as well as PCR-based molecular techniques for
identification of dermatophyte species from superficial mycoses in human patients. The following study with the objectives given below has therefore been proposed.

1.1 Objectives

• Collection and isolation of dermatophytes from human clinical cases.
• Identification and phenotypic characterization of isolates of dermatophytes.
• PCR based identification of dermatophyte species.
• Determination of susceptibilities of the isolates to antifungal agents (itraconazole, terbinafine and ketoconazole).