1. Introduction

In the past few decades, the incidence of microbial skin infections especially dermatophyte infections has increased worldwide (Straten et al., 2003; Sharma and Borthakur, 2007; Jain et al., 2008). The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair, and nails) of humans as well as other animals to produce superficial infections known as dermatophytoses, which is commonly referred to as ringworm (Fig. 1). The dermatophyte infections have also been named by appending the Latin term designating the infected body site after the word tinea. For example, the clinical manifestations are termed as tinea capitis (ringworm of the scalp), tinea barbae (beard and mustache), tinea corporis (glabrous skin), tinea cruris (groin), tinea manuum (hand), tinea pedis (feet) and tinea unguium (nails).

Fig. 1 Dermatophyte infections

Dermatophytoses are of serious concern not only from medical but also from social and economic point of view. The infections are highly contagious and cause instant itching, extreme discomfort and pose a critical health problem. The causal organisms are transmitted either by direct contact with the infected host or direct or indirect contact with infected exfoliated skin, combs, hair brushes, clothing, furniture, theatre seats, caps, hotel
rugs etc. Immunocompromised persons have higher risk of fungal skin infections (Straten et al., 2003; Weitzman and Summerbell, 1995). There is an increased susceptibility to infections where there are excessive temperature and humidity and when there is a pre-existing injury in the skin such as scars, burns etc. These diseases are prevalent throughout the tropical and subtropical regions of the world. They are of serious health concern, not only because they are life-threatening but also due to the extreme discomfort, stress, pain and ugliness they cause. The incidence of such infections has been reported from various parts of India as well as other countries (Jain et al., 2008; Prasad et al., 2005; Kannan et al., 2006; Straten et al., 2003; Nweze, 2010).

The Northeast region of India is highly conducive for the growth of large number of pathogenic microorganisms including dermatophytes. The hot and humid climate of this region is highly favourable for proliferation, survival and longevity of dermatophytes. It is a serious health problem of sociologically and economically backward common people of Northeast India. Indian soldiers working in hostile environment of forests of the remote border areas are more prone to such skin infections. This hampers the efficiency of the defence forces. Investigations on the occurrence of skin diseases in some parts of Northeast India revealed that the dermatophytoses were predominant among the fungal skin diseases reported from this region (Jaiswal, 2002; Sharma and Borthakur, 2007; Devi and Zamzachin, 2006 and Das, 2003).

Treatment of dermatophytoses presents great difficulties, as fungi and their hosts both being eukaryotic, makes it more difficult to select intracellular drug target, specific for
pathogen and making no deleterious effect to host. The major classes of synthetic antifungal compounds, presently available, are the polyene macrolides (e.g. amphotericin B), the azoles (e.g. ketoconazole, miconazole, clotrimazole, fluconazole, itraconazole etc.) and the allylamines (e.g. terbinafine). Other synthetic antifungals include griseofulvin and flucytosine. The majority of these antifungals, however, have several drawbacks as regards to their toxicity, efficacy and cost. The emergence of resistant strains due to frequent use of these chemicals is another serious problem. The strong biological variability of the dermatophytoses has so far prevented the development of a single effective drug against all these diseases. Concerns have already been raised about the environmental impact as well as the potential health risk related to the use of these synthetic compounds. Hence, there is an urgent need for the development of new antimycotic drugs, which can take care of these problems.

Nature has been the source for medicines long before the advent of present technological era. Plants are the most important resources on the earth, which are of critical importance for health security of the present and future generations. Traditional folk medicines are the best examples of this (Li et al., 2008; Khan et al., 2009; Phongpaichit et al., 2005; Seneviratne et al., 2007; Webster et al., 2008). The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand (Hoareau and DaSilva, 1999). In some Asian and African countries 80% of the population depends on traditional medicine for their primary health care (Anonymous, 2008). The beneficial role of herbs in therapeutic treatment is an important breakthrough in the history of mankind. Consequently, in the recent years, there has been a rapid development in high throughput
screening methods for biological activities of herbal prescriptions (Cowan, 1999; Ficker et al., 2003; Svetaz et al., 2010; Katiyar and Sharma, 2005). The traditional medicine is gaining importance in modern medicines because this makes the task of searching a new potential medicinal plant easier. Revival of interest in plant derived drugs is also due to the current widespread belief that ‘Green Medicines’ are safe. As a consequence, there is an increasing trend, world wide to integrate herbal medicines with health care. It is, therefore, pertinent to test plants that are used traditionally for their medicinal and antifungal activities for exploring the scientific rationale and also to search for a potent source for antimycotic drug. (Li et al., 2008; Phongpaichit et al., 2005; Seneviratne et al., 2007; Webster et al., 2008).

The Northeast India, a part of the Indo-Burma hotspot, is one of the mega hotspots of biodiversity in the world. It contributes more than 50% of plant species to the total Indian flora. A WWF report on ‘Biodiversity Assessment in the North Bank Landscape’ emphasized that forests of Assam and Arunachal Pradesh are amongst the biodiversity rich areas of the world. Another study carried out in the foothills of the Himalayas of Northeast India recorded 107 species of plants in a 200 sq m plot in the North Bank Landscape. Dr. Andrew Gillison of WWF termed the North Bank Landscape as "the jewel in the crown of Indian forests" (Anonymous, 2004; Banerjee, 2007). Approximately 70% of about 4000 plant species that are used for medicinal purpose in India are found in the northeast. This region is a center of species diversity of Indian Piper (Rahiman and Nair, 1994). The border of Assam and Arunachal Pradesh, that extends along the foothills of the Himalayan range harbours unique medicinal plants, many of them are still
unexplored. This region is also experiencing habitat loss and habitat fragmentation that ultimately would result in biodiversity loss (Yumnam, 2008). Many of the important medicinal plants might also be lost even before they are explored. Many of these plants are being used by the local people living in that area for treatment of various ailments. The region, with its rich plant genetic resources and traditional knowledge acts as key components for bioprospection of medicinal plants for therapeutics, provides unique opportunity to target plants with antimycotic properties. Unfortunately, due to reasons like inaccessibility of some tough terrains, the flora of this region has not so far been explored properly.

The use of plants as medicines involves isolation of active compounds. This began with the isolation of morphine from opium in the early 19th century (Huxtable and Schwarz, 2001). Of the several hundred thousand plant species around the globe, only a small proportion has so far been investigated from phytochemical and pharmacological point of view. Plants contain thousands of constituents and are a valuable source of new and biologically active molecules. The ultimate success of an investigation leading to discovery of bioactive plant constituents depends on selection of appropriate plant material, suitable biological assays and chemical screening methods. The process that leads from the plant to a pharmacologically active and pure constituent(s) is very long and tedious, and requires a multidisciplinary approach. Biological and chemical screenings are complementary approaches for the rapid detection and isolation of interesting new bioactive plant constituents. Biological screening followed by activity-guided fractionation has been used successfully for the discovery of antifungal, antibacterial,
antimalarial bioactive compounds (Hostettmann, 1999). Suitable methods and proper performance of the bioassay are crucial for monitoring the fractionation and isolation of bioactive components. Various chromatographic, spectroscopic and chemical methods were employed for the isolation and identification of bioactive components.

Medicinal plants and products derived from them have a unique place in both preventive as well as curative medicine, as these plants produce a remarkably diverse array of organic compounds, called secondary metabolites. The majority of natural products derived from medicinal plants are secondary metabolites viz., terpenoids, steroids, cardenolides, quinine lignans, flavonoids or alkaloids. The active molecules isolated from traditional medicinal plants might provide valuable drugs. Many active compounds from traditional medicine sources could serve as good scaffolds for rational drug design. Challenges in bioassay screening and identification of active components remain an important issue in the future of drug discovery from medicinal plants.

Another important aspect, in herbal medicines is the standardization and quality control with proper integration of modern scientific techniques and traditional knowledge. In the current scenario, medicinal plants and their genes are being increasingly recognized as resources of high economic value. Various DNA-based methods for the authentication of plant species, particularly those, which are morphologically indistinguishable and also in detection of adulteration in medicinal plants have been published. With the evolution of these molecular approaches as role of pharmacognosy is likely to be more challenging in forthcoming years. The spectacular advances in the field of molecular biology in recent
years have enabled us to properly document herbs by using DNA fingerprinting technique that is less time consuming and more authentic. This would also ensure the property rights of our country on the valuable medicinal germplasms in the post GATT scenario. Moreover, extensive information on DNA profiles of medicinal plants is not available except for few species. Random amplified polymorphic DNA (RAPD) technique is a robust and one of the most commonly used DNA techniques for primary assay of genomic DNA. One of the most important applications of RAPD is the identification of plant species with no prior sequence data or clarification of the differences between species.

Considering the necessity of bioprospecting medicinal plants using modern techniques that would be the key in developing novel and effective antifungal agents in fighting fungal skin diseases in general and dermatophytoses in particular, the present investigation was designed, focusing on the following objectives:


2. *In vitro* evaluation of extracts of selected plants as well as fractions thereof for antidermatophytic activity.

3. Phytochemical characterization of effective antidermatophytic components.