INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Grabley and Thiericke, 1999; Owolabi *et. al.*, 2007).

Historically, a large portion of the world's medicine has been derived from plants. Natural products chemistry actually began with the work of Serturner, who first isolated morphine from Opium. This, in turn, was obtained from opium poppy (*Papaver somniferum*) by processes that have been used for over 5000 years. Many such similar developments followed. Quinine from Cinchona tree had its origin in the royal households of the South American Incas. Before the first European explorers arrived, the native people of the Americas had developed complex medical systems replete with diagnosis and treatment of physical as well as spiritual illnesses (Patwardhan *et. al.*, 2004). Salicin from *Salix alba* which on oxidation was converted to salicylic acid is the active ingredient in aspirin (Piria, 1938; Jeffreys, 2005) which has been used for millennia as an effective pain reliever and fever reducer. Drugs such as aspirin, vincristine, vinblastine, cocaine, digitoxin and morphine are also isolated from plants (Newman *et. al.*, 2000; Butler, 2004; Gilani and Rahman, 2005).

In the recent years, research on medicinal plants has attracted a lot of attentions globally. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found to have antimicrobial properties *in vitro* (Dahanukar *et. al.,* 2000). The so-called secondary metabolites (Evans *et. al.*, 1986) can be classified as chemotherapeutic and antimicrobial (Purohit and Mathur, 1999). The use of plant extracts and phytochemical both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal

plants (Nascimento *et. al.*, 1990; Alonso *et. al.*, 1995; Islam *et. al.*, 2008; Manjulata *et. al.*, 2012).

'Doctrine of signatures' written in 15th century stated "A plant could treat a disease it most looked like", This formed the basis of phyto-therapeutics in traditional system of medicines like Traditional Indian Medicine (TIM) or Ayurveda, Traditional Chinese Medicine (TCM) and Western Medical Herbalism (Bown,2001). The ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in the treatment of various diseases (Abu-Shanab *et. al.,* 2004).

According to a report issued by the World Health Organization (WHO), plant species that were used for medicinal purposes are about 20,000 (Vartia, 1973). Current estimates indicate that about 80% of people in developing countries still rely on traditional medicine based largely on various species of plants and animals for their primary healthcare (Goodwin *et. al.*, 1997).

For example, the use of bearberry (*Arctostaphylos uvaursi*) and cranberry juice (*Vaccinum macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are redescribed as broad-spectrum antimicrobial agents (Rios and Recio, 2005).

Since the discovery of penicillin (1929) and its use in chemotherapy in 1941 as a response to the great fatalities in the Second World War, a great number of important antibiotics have been found (El- Bana, 2007). The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols, which are water, ethanol, chloroform, methanol and butanol soluble compounds. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus (Elmastal *et. al.,* 2005).

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents (Gomathi *et. al.*, 2011). The search for novel bioactive compounds from natural resources to improve pharmaceutical, cosmetic and agriculture applications is an ancient practice and currently it is regaining more rapid importance. Most citrus and dried fruits, cruciferous vegetables, garlic, onions, carrots, tomatoes, sweet potatoes, sesame and olive oil are rich sources of antioxidants. Antioxidants are molecules that can delay or prevent an oxidative reaction (Velioglu *et. al.*, 1998) catalyzed by free radicals.

Indian medicinal plants represent a rich source of antibacterial agents (Mahesh and Satish, 2008). To the botanist the Himalayas are the treasure-house for not only the study of the plants growing there but also for plants new to science and particularly those plants which are of great medicinal and economic value for the welfare of mankind.

Lichens are another type of organisms that may hold the potential for medical exploration. The word lichen is derived from Greek word "Leprous" and refers to use of lichens in treating skin diseases due to peeling–skin appearance. Lichens comprise a unique group that consists of two unrelated organism, a fungus and an alga, growing together in a symbiosis. Lichens with blue green algae symbionts, contribute significantly for forest nitrogen fixation. They are distributed universally and are occurring in varied climatic conditions ranging from the poles to the tropics. They may look like crust, spreading rapidly over the surface (crustose) or leafy and loosely attached to the surface (foliose) and branched and shrubby, hanging from tree twigs or branches, with a single attachment (fruticose). Besides many other uses, lichens are also used as pollution monitors (Nash and Wirth, 1988; Richardson, 1992; Stolte *et. al.*, 1993; Slack, 1998; Garty, *et. al.*, 2000; Nash and Gries, 2002; Kumar, 2009).

In the folklore of many European countries, lichens were used as a remedy for pulmonary tuberculosis and in treatment of wounds and disorders. These medicinal uses to some extent been confirmed by studies which showed that many lichen metabolites such as depsides, depsidones and usnic acid are active against mycobacteria and gram positive bacteria (Vartia,1973).

Lichens like *Lobaria pulmonaria* (Stictaceae) and *Parmelia sulcata* (Parmeliaceae) have been used in the treatment of pulmonary and cranial diseases, respectively. Similarly, *Xanthoria parietina* (Lobariaceae), being yellow, was used to cure jaundice (Bown, 2001).

Various biological activities of some lichens are known, such as: antimicrobial, antiviral, anti-tumor, anti-inflammatory, analgesic, antipyretic, antiproliferative and antiprotozoal (Lawrey, 1986; Huneck, 1999;Halama and Van, 2004). The lichen compounds are not an exception in this field. Currently the interest on the lichen secondary compound is increasing because of ineffectiveness of some known previously reliable drugs (Huneck, 1999).

A lot of attention has been paid to lichens as a source of natural antioxidants (Behera *et. al.*, 2006; Gulluce *et al.*, 2006; Kinoshita *et. al.,* 2010; Rankovic *et. al.*, 2010; Dzomba *et. al.*, 2012). Antioxidant activity of some other lichen was studied by other researchers. For example, Gulcin *et al.*, (2002) reported that the aqueous extracts of *Cetraria islandica* had a strong antioxidant activity. Similar results found by Behera *et. al.*, (2005) for different extracts from the lichen *Usnea ghattensis*. Antioxidant activity for the extracts of the lichen *Parmotrema pseudotinctorum* and *Ramalina hossei* was also documented (Kekuda *et. al.*, 2009).

India is a rich center of lichen diversity contributing of about 15% of the 13,500 species of lichens so far recorded in the world (Negi, 2000). In India parmeloid lichens are extensively used in traditional medicine to treat several diseases and disorders e.g., headache, skin diseases, urinary trouble, boils, vomiting, diarrhoea, dysentery, heart trouble, cough, fever, leprosy and as blood purifier (Chandra and Singh, 1971).

Lichens of Eastern Himalayan regions, particularly of Darjeeling District have been studied extensively by Chopra (1934) and Awasthi and Agarwal (1970) from taxonomic point of view, but the antimicrobial and antioxidant properties of these lichens have not yet been explored to that extent. Gupta and Paul (1995) reported antimicrobial property of *Usnea floria, Physcia* sp*, Usnea pendulata, Cladonia cristatella, Parmelia perforata* and *Ramalina calicaris* collected from Darjeeling Hills against *Bacillus megaterium, Staphylococcus aureus.* In another study from Darjeeling hills

Ray *et. al.*, (2003) screened the extract of *Usnea articulate, Ramalina jamesii* and *Parmelia tinctorum* against both Gram positive and negative bacteria and antimicrobial activity was reported to most of the tested micro organisms. The extracts were also found to be inhibitor of protein synthesis, energy metabolism and growth of selected bacteria.

In view of the limited information on *in vitro* activity of lichen members and the abundant distribution of lichens in the varied locations of Darjeeling Hills, the present study includes determination of potential pharmaceutically relevant antimicrobial and antioxidant activities of selected lichen species from profusely grown locations of Darjeeling Hills.

Objectives

The study was carried out with following objectives:

- ✓ To study the antimicrobial property of some high altitude lichen members of Darjeeling Hills.
- ✓ To study the antioxidant property of some high altitude lichen members of Darjeeling Hills.
- ✓ To study the synergistic antimicrobial and antioxidant activity of such lichens in combination with selected local medicinal plants.
- ✓ To identify active principle compounds from the lichens showing potential antimicrobial and antioxidant activity.