CHAPTER 1

INTRODUCTION

1.1 General account

Lichen, the complex life form is rather a symbiotic association of autotrophic microalgae or cyanobacteria and heterotrophic fungi. It is known to be the earliest colonizers of terrestrial habitats of worldwide distribution and dominates 8% of the earth's land surface (Asplund and Wardle, 2016). It has the ability to grow on any substratum in any geographical region and can survive even in extremes of environment with temperature exceeding 55° C in hottest desert and −50° C in Antarctica (Mitrović *et al*., 2011; Calcott *et al*., 2018; Sonia and Androsova, 2020). Major part of the thallus (90%) is made up of mycobiont with the remaining composed of photobiont. Whatever visible externally is fungal that envelopes the algae within. Hence it follows fungal classification. The three main groups of lichen, crustose, foliose and fruticose have been divided based on their morphological appearance. The crustose lichen is tightly adhered with the substratum and lacks lower cortex and rhizines; in foliose lichen, the thallus is loosely attached to substratum and is known as leafy lichen; the thallus of fruticose lichen is attached to substratum at one point but the other part remains hanging or erect, looks as a small shrub or bushy. Other intermediate growth forms include leprose, placodioid, squamulose and dimorphic lichens (Nayaka, 2014). About, 25,000 species of lichen are distributed worldwide (Soundararajan *et al*., 2019); of which India represents 3028 lichen taxa belonging to 469 genera and 88 families (ILS eLetter 2021), and currently Assam records 657 species of lichen under 146 genera and 41 families (Gogoi *et al*., 2022); however, the number of lichen species is increasing with more explorations.

Lichens are known as bio-indicators of air quality as they are very sensitive to air pollution and climate change and have been utilized for biomonitoring studies globally (Shukla *et al*., 2014). Since ancient times, lichens have been used by ethnic groups spread globally for various purposes, the most common of which are for dyeing, in medicine, as food, decorative, brewing, distilling, cosmetic and perfumery (Upreti *et al*., 2013). Powdered lichens are being used as a major ingredient of common masala in India such as meat masala, garam masala and shambar masala (Upreti *et al*., 2005). These significant

properties of lichen have been proven by several pharmacological studies and it has now been considered as a potential alternative therapy for modern diseases (Zambare and Christoper, 2012; Kosanić and Ranković, 2015; Elkhateeb and Deba, 2019; Fang *et al*., 2020). Lichenized fungi synthesize wide variety of secondary metabolites with different bioactive properties which are unique to the group (Crawford, 2015; Sweidan *et al*., 2017; Ponmurugan and Ayyappadasan, 2017). These substances keep them protected from abiotic stress and biological attack (Ramakrishna and Ravishankar, 2011; Gómez-Serranillos *et al*., 2014). Lichen produces more than 1050 secondary metabolites, majority of which are produced through the polyketide pathway, and primarily consist of monocyclic and bicyclic phenols attached by an ester bond (Stocker-Wörgötter, 2008; Karagöz *et al*., 2009). These lichen substances are usually traced on the surface of mycelium cells and are mostly water-insoluble (Zhao *et al*., 2021). Fairly few lichen substances have been thoroughly monitored for biological activities and therapeutic potential; this is primarily due to slow growth rate and unavailability of having them in large quantities and adequate purities for structural elucidation and pharmacological tests (Kosanić *et al*., 2014; Crawford, 2015; Manojlović *et al*., 2021; Pham *et al*., 2021; Gandhi *et al*., 2022). Similarly, the effectiveness of lichen substances has been examined for antioxidant potentials against oxidative damage with the help of oxidative stress markers such as superoxide dismutase or malodialdehyde (Paluszczak *et al*., 2018). Antioxidants protect the human body from oxidative damage caused by free radicals that deters the development of many chronic diseases including cancer, heart, neurogenerative diseases (Kim *et al*., 2015). This natural product can revive the behavior of cancer cells by shifting their redox environment as well as reduce their genetic unpredictability and thus may be considered useful in cancer treatment (Nguyen-Trinh *et al*., 2022). Protection from carcinogenesis or mutagenesis is due to numerous activities such as antioxidant, cytotoxic, pro-apoptotic, anti-proliferative, anti-migrative, anti-invasive, and anti-tumorigenic properties in lichen substances (Zambare and Christopher, 2012; Ristić *et al*., 2016; Studzińska-Sroka *et al*., 2016; Nguyen *et al*., 2019). The cytotoxic ability of lichen as observed in different cancer cell lines reveals higher cytotoxic activity in cancer cells than in non-cancer cells (Zambare and Christopher, 2012; Nguyen *et al*., 2019). Strong

cytotoxic ability of lichen against cancer cells is mediated through apoptosis, inhibition of angiogenesis, necrosis or autophagy together with cell cycle arrest G2/M, S, or G0/G1 phases (EI-Garawani *et al*., 2019; Yurdacan *et al*., 2019). Cancer is a life-threatening, complex disease due to its invasiveness and metastatic behavior. Earlier reports in 1990, reveals a progressive trend in the rate of cancer cases with lung, colorectal and stomach as the most common forms of cancer (Parkin *et al*., 2001). In between 2012 and 2020 the rate of cancer cases has been increased by 37% to reach 19.3 million (Sung *et al*., 2021; Ferlay *et al*., 2015). US alone has crossed 1.8 million new cases of cancer representing 9% of the global cancer occurrence in 2020 (Siegel *et al*., 2020). Among them, breast cancer is one of the most common lethal types of cancer prevalent in women and leads to the deaths of 1 on every 10 women diagnosed with cancer (Azamjah *et al*., 2019; Alkabban and Ferguson, 2021). In 2020, of the total 2.3 million women detected with breast cancer globally, 685,000 died due to the disease (WHO, 2022). The most conservative methods for cancer treatment include surgery, radiotherapy, and chemotherapy (Sharifi-Rad *et al*., 2019); of which chemotherapy serves as the most effective and preferred. But with time, resistance to drugs limits its effectiveness in maximum cases (Ghate *et al*., 2013). Sometimes, synthetic drugs are incapable of differentiating between normal and cancerous cells (Ghate *et al*., 2013). In this regard, there is always an urge to find new chemotherapeutic agents of natural origin with minimal side effects (Cardile *et al*., 2017; Studzińska-Sroka *et al*., 2021). Around 60% of all natural products are known as pharmaceutical agents; more than half of all these approved drugs are being used for cancer treatment (Newman and Cragg, 2020).

These specific natural compounds play significant roles in modern drug development, especially for antimicrobial agents, due to a significant emergence of multidrug-resistant (MDR) pathogenic microorganisms. Evolution of multidrug resistance in commensal bacteria has become a prominent public health concern and long-term use of synthetic drugs often causes numerous side effects (Karaman *et al*., 2003; Jacopin *et al*., 2020). The main reasons for the development of drug resistant pathogens are misuse and overuse of antimicrobial drug (WHO, 2017). Unlike synthetic drugs, natural bioactive products have beneficial effect on the whole organism without causing unwanted effects (Ranković *et*

al., 2011). The progressive loss of efficacy in conventional anti-infective treatments represents a high task for herbal medicine to develop drugs with a broad-spectrum antimicrobial activity with lower side effects, that can control and prevent various human, animal and plant diseases (Karaman *et al*., 2003; Anand *et al*., 2020).

1.2 Choices of the present work

In forest ecosystems, lichens are one of the major components and their growing richness and distribution are influenced by ecological and environmental variables. Accurate species level identification of biodiversity is crucial for ecology, evolution, and conservation biology, and it determines the effects of global change on ecosystems. However, it is still challenging to quantify biodiversity, particularly for geographical regions that receive less attention. The lichen biota of Kokrajhar district have been explored sparsely but several taxonomic groups remain poorly known. Few studies with fragmentary records have been devoted to investigate the lichen diversity of the region.

Ultapani Forest Range is well known for its richness in biodiversity. The vegetation of forest is distinctive with unique semi-evergreen and moist deciduous forest. It is home to rare, endangered, threatened species of plants; and orchids are another asset to this forest. Despite the existence of a healthy forest, dearth research has been done on lichen of UFR. Therefore, the quest of lichen diversity that correspond to their distribution was primarily based on review of few published information.

Many lichen species are engaged in a variety of biological activities as a result of naturally occurring chemical combinations and their interactions. Finding novel chemical compounds that act as antioxidants and defend human body against free radicals is focus of current medical research. Natural antioxidants are used to treat cancer because of its ability to alter the behaviour of cancer cells by changing their redox environment, which lessens their genetic unpredictability (Nguyen-Trinh *et al*., 2022). Several strategies were employed in fighting cancer; the importance of chemotherapy is specified most of the time, as it is showing effectiveness against many cancer types. But resistance to synthetic drugs limits successful results in most cases. It is also well known that increased and

uninhibited long-term use of synthetic drugs frequently results in resistance and overflowing negative effects, and these have led to the discovery of new sources of natural products for control and prevention of various diseases related to humans, animals and plants.

Discovery of antibiotics have developed the field of medicine as they protected uncounF lives from infectious diseases caused by pathogenic microbes. Despite the use of numerous antibiotics, the ongoing conflict between human and pathogens will never end and excessive use of antibiotics can occasionally result in emergence of bacterial strains that are resistant to them. These resistant strains create massive complications in nosocomial and community settings. It might be challenging to treat diseases often caused by these strains; consequently, there is always a need to find new strategies to invent novel and effective antibiotics of natural origin. Thus, natural product exhibits as one of major alternatives for developing antimicrobial agents.

The species, *Anzia ornatoides* was selected for the study of biological activity with the following facts:

1. Unavailability of records on the study of human pathogens and cancer cells involving the lichen species, *Anzia ornatoides*.

2. Biomass of a lichen is very meagre so availability of the species is an important factor for carrying out such studies.

3. The species possesses important phytoconstituents such as alkaloid, carbohydrate, flavonoid, glycoside, phenol, and saponin.

3. Different secondary substances like atranorin, lobaric, sublobaric, stictic, protocetraric, and fumaprotocetraric acids are found in *A. ornatoides*, are known to have biological activity.

In light of the above facts, the topic entitled, “Lichen diversity of Ultapani Forest Range, Manas Biosphere Reserve, Assam and biological activity of selected species”, was chosen for the present study.

1.3 Aim and objectives

The present study was undertaken to comprehend the lichen diversity of Ultapani Forest Range, Manas Biosphere Reserve, Assam and to enhance the knowledge of biological activity of lichen species.

The objectives of the study include:

1. To enumerate lichen diversity of Ultapani Forest Range
2. To investigate phytochemical analysis and cytotoxic activity of *Anzia ornatoides*
3. To determine antimicrobial potential of *Anzia ornatoides*