1.1 General Introduction

Eastern Himalayan region is a biodiversity hot spot. The region is dominated with a temperate broadleaf forest ecoregion found in the mid elevations of the eastern Himalayas, including parts of Bhutan, Nepal, India, China and Myanmar. These forests have an exceptional high diversity of flora and fauna. The lifestyle of the region is dominated by agriculture and animal husbandry.

A diverse array of fungal species from different classes is identified to infect insects, exhibiting an extensive range of adaptations and infecting capacities, including obligate and facultative pathogens (Gebremariam et al., 2022). There are approximately 750 fungal species known to cause infections in insects and mites (Sinha et al., 2016). While this group collectively targets a wide-ranging insect and mite species, individual fungal species and strains exhibit high specificity (Quesada-Moraga et al., 2022). Entomopathogenic fungi form a diverse group with the capability to infect and eliminate insects, arthropods, and other invertebrates. They have important and decisive role in controlling arthropod and pest populations in natural habitat. Among them, *Cordyceps* spp. stands out as a cluster of entomopathogenic fungi well-known for their distinctive morphology, diversity, and medicinal properties.

There are about 750 species of *Cordyceps* that have been documented. The abundance of *Cordyceps* spp. is closely linked to the abundance of their hosts, primarily thriving in high elevations ranging from 3,800 to 4,000 meters above sea level (Zhang et al., 2020). Consequently, *Cordyceps* spp. has been identified in diverse regions across Europe, Asia and North America, particularly in countries such as Bhutan, Japan, China, Nepal, Korea, Thailand and Vietnam (Das et al 2021). In India, it is predominantly found in subalpine regions, specifically in the Garhwal and Kumaunregion of Uttarakhand at higher altitudes (Thakur, 2020). It is also found in Eastern Himalayan region of India in Sikkim and Arunachal Pradesh (Biswa et al., 2021).

Additionally, reports indicate the presence of species like *C. gunnii* (Berk.) Berk. in Australia (Nxumalo et al., 2020). The metabolite structure of *Cordyceps* species gives them resistance against low temperatures, low oxygen levels, and UV radiation, which are common at high elevations. Moreover, over the course of its three-phase life cycle infection, parasitic behavior, and saprophytism—these unusual medicinal mushroom spreads by rain, air, and insects (Pal & Misra, 2018). *Cordyceps* spp. contaminates the larva via ascospores distributed through atmosphere from fruiting body to infect

throughout summer and early autumn, and then germinates to form new fruiting body (Das et al., 2021).

The parasitic phase occurs past infection, and *Cordyceps* spp. feeds on the host's bowels (Das et al., 2021). In winter, fungus cells broaden throughout the organism and expand quickly, devouring the larva's interior organs while leaving its outer shell intact. Following which, the fungi transform to a white mass inside the host larva called the endosclerotium (Das et al., 2021). Throughout this development, the mushroom encounters challenging environmental conditions, enduring snowfall and cold temperatures. As spring unfolds and external temperatures rise, the endosclerotium germinates, protruding through the host's oral cavity (Das et al., 2021). Maturation takes place in the summer, giving rise to fruiting and initiating the dispersion of ascospores (saprophytic stage) (Das et al., 2021).

The summer season serves as the collection period for the fungus traditionally undertaken by villagers engaged in grazing practices, particularly those tending to yaks, in alpine regions (Baral, 2017). Over the months, these gatherers remain in these high-altitude areas, looking after their animals and harvesting the fungus alongside other medicinal plants (Chaubey et al., 2019). Traditional healers, frequently visit these regions for mushroom collection, accumulate the dehydrated material for future use. The therapeutic significance of *Cordyceps* spp. has resulted in enhanced popularity, accompanied by concerns about over-harvesting leading to the depletion of wild species. Consequently, since the 1970s, various scientists have explored options for fermenting and cultivating isolated fungi. Recognized for their healing effects and curative activities for numerous years, *Cordyceps* spp. have found applications in folk medicine for treating various diseases (Das et al., 2021).

These spores of fungi initiate infection by germinating on the host's surface and subsequently growing on its body. Duration of death varies between 4 and 10 days, contingent on type and the amount of infecting spores (Sinha et al., 2016). Following the host's demise, the fungus generates thousands of new spores on the deceased body, which disperse and continue their life cycle by infecting new hosts (Sinha et al., 2016).

1.2 *Cordyceps* spp.

The first report comes from the herders from high altitude region of Tibetan and Himalayan region. They found that the animals grazing in the region had high vigor due

to consumption of a fungi; *Cordyceps* spp. it was then utilised for treatment of different ailments through traditional system of healing. Then the further scientific study revealed the different medicinal potentials of the *Cordyceps* spp. (Ghanshyam & Manvitha, 2017)

Tibetan medicine acknowledges *Cordyceps* spp. as an essential source of vital energy, as reported by users. *Cordyceps* spp. belongs to taxonomic classifications including Clavicepitaceae, Pyrenomycetes, Ascomycota, and Hypocreales, encompassing over 700 recognized species (Das et al., 2021). The "*Cordyceps*" comes from the Greek word "kordyle," meaning "club," and the Latin term "ceps," signifying "head" (Das et al., 2021). The behavior of *Cordyceps* spp. is intriguing, infiltrating arthropods, insects, other fungi, and proficiently avoiding the host's immune system (Das et al., 2021). This synchronization with the host's life cycle aims at ensuring survival and proliferation, leading to the production of various secondary metabolites, including exopolysaccharides, cordymin, adenosine, gamma-aminobutyric acid (GABA), cordycepin, cordysinin A, B, C, D, E, guanosine, among others (Cao et al., 2020).

Cordyceps encompasses diverse species, each endowed with valuable properties, such as anti-cancer, anti-aging, hypolipidemic, anti-inflammatory, anti-thrombotic, anti-hypertensive, steroidogenesis, apoptosis induction, anti-proliferative, anti-arteriosclerosis, anti-metastasis, antidiabetic, antimalarial, immunomodulatory, anti-asthmatic, hypoglycemic, and antifungal effects, along with antioxidant, anti-fibrotic, and anti-angiogenic properties (Zhang et al., 2019). Expression of those properties is dependent on concentration and no detrimental effect was reported in most cases (Das et al., 2021).

Notably, *Cordyceps* spp. harbors diverse compounds capable of enhancing the immune system's response and regulating its overactive reactions. While much information about the impact on immune system by *Cordyceps* was inferred from studies predominantly focused on cancer, it has been identified to improve the generation of interleukins IL-6, IL-10, IL-8, (IL)-1β, IL-2, TNF-α and IL-12 (Das et al., 2021). It triggers the release of nitric oxide (NO), and triggers an inflammatory reaction via the MAPK pathways (Olatunji et al., 2018). Additionally, it exhibits synergistic effects with interferon (INF)-γ in cytokine production (Das et al.,2021). These effects make *Cordyceps* spp. particularly attractive for applications where immune system stimulation is desired. Consequently, this review compiles and evaluates the present understanding of the immunostimulatory characteristics of *Cordyceps* spp.

Cordyceps spp. encompasses a genus of parasitic fungi recognized for its various biological activities and potential health benefits. Thriving in mountainous regions, particularly in Asia, these fungi parasitize insects or arthropods, giving rise to unique fungal fruiting bodies. *Cordyceps* spp., such as *Cordyceps sinensis* and *Cordyceps militaris*, have a rich history in traditional Chinese medicine, valued for their purported medicinal properties. Packed with bioactive compounds like polysaccharides, nucleosides, peptides, and sterols, *Cordyceps* species exhibit immunomodulatory, anti-inflammatory, antioxidant, and potentially anti-tumor properties. Recognized as adaptogens, these fungi aid the body in adapting to stress and restoring balance (Tareq et al.,2023). *Cordyceps* supplements, popular in sports and fitness circles, are believed to enhance endurance and aerobic capacity. Despite promising research, further studies are necessary to comprehensively understand the varied species within the genus and establish the full extent of their effectiveness for diverse health conditions. As with any supplement, consulting a healthcare professional is advisable before incorporating *Cordyceps* or other herbal products, especially for individuals with underlying health conditions or taking medications.

1.3 Characteristics, Habitat and distribution of *Cordyceps* spp.

Among the vast range of mushroom species identified within the *Cordyceps* genus, about 20 species are identified to parasitize the Elaphomyces genus. The other species target various host belonging to classes such as Hymenoptera, Arachnida, Isoptera, Hemiptera, Lepidoptera and Coleoptera (Das et al., 2021). This diverse range of species encompasses notable members such as *C. minuta*, *C. gracilis*, *C. scarabaeicola*, *C. sphecocephala*, *C. konnoana*, *C. sobolifera*, *C. nutans*, *C. gunnii*, *C. agriota*, *C. ophioglossoides*, *C. tuberculate*, *C. canadensis*, *C. subsessilis*, *C. myrmecophila*, *C. cicadae*, *C. militaris*, *C. ishikariensis*, *C. nigrella*, *C. pruinosa*, *C. sinensis*, and *C. tricentri*, among others, among others (Deshmukh et al., 2020).

These *Cordyceps* species exhibit diverse characteristics, including pharmaceutical properties, rendering them particularly attractive in traditional Chinese medicine (TCM) since the 1990s, with *C. sinensis* standing out as the most extensively researched and applied (Das et al.,2021).

1.4 Traditional uses of *Cordyceps* spp.

Over the course of numerous centuries, *Cordyceps* has secured a prominent position in TCM as a tonic, addressing a range of ailments such as liver or renal issues, respiratory diseases, hyperglycemia, and ailments related to tumors or cancer (Cunningham & Long, 2019). Additionally, *Cordyceps* spp. has been utilized to boost energy levels, improve endurance, enhance aerobic capacity, and strengthen cellular immunity. The formal recognition of *Cordyceps* in traditional medicine dates to 1964 as a drug in the Chinese Pharmacopoeia (Deng et al., 2020).

Notably, *Cordyceps militaris* and *Cordyceps sinensis* is positioned as the most used species. In specific regions, including Nepal, China, Bhutan, the Tibetan plateau, and India, the amount and direction of use of *C. sinensis* depend on the expertise and proficiency of traditional practitioners, often determined through a trial-and-error approach Yan et al., 2017). For example, in certain communities, the fungus is dissolved in alcohol, hot water or milk, serving various purposes such as enhancing desire and sexual potency or acting as a morning tonic (Holliday, 2017). The synergistic effects of *Cordyceps* spp. along with additional bioactive molecules have been reported.

Traditional healers use *Cordyceps* spp. along with ginseng root and taxus leaf for cancer treatment. Additionally, *C. sinensis* is acknowledged as a nutritious food in Chinese culture, likely because of its composition rich in vitamins B1, B2, B12, K, essential amino acids carbohydrates, and other constituents. Notably, this fungal species serves as a dietary supplement compliant with the standards of the U.S. FDA, resulting in its widespread global demand for *cordyceps* (Wei et al., 2017). Conversely, *Cordyceps* spp. demonstrated effectiveness in therapy for exhaustion and weakness, alleviating signs of high elevation sickness and imparting an energy increase to individuals. In elderly individuals, reports indicate diminished aches and pains (Das et al., 2021).

Experts in TCM advocate regular consumption of *C. sinensis* to prevent colds, infections and flus, citing its efficacy in reducing coughs, phlegm, asthma, and bronchial diseases (Charen & Harbord, 2020). Subsequently, *Cordyceps* spp. has been used to treat fibrosis of the lungs, especially among patients with serious acute respiratory syndrome (SARS), in accordance with TCM principles that link these characteristics to *C. sinensis* ability to sustain lung tissue (Chen et al., 2020).

The positive influence of *Cordyceps* spp. has been observable in sports person, with documented improvements in energy levels attributed to heightened cellular ATP levels, resulting in enhanced energy release in muscle. Proportionate to *C. sinensis*, *C. militaris* (from in China, East Asia, Japan, and Korea) encompass its roles as an energy booster, aphrodisiac and a therapy for respiratory conditions (Zeng et al., 2019). Additionally, this species is associated with antifungal, immune-protective, hypoglycemic, antioxidant, antibacterial, antitumor, and anti-inflammatory properties, solidifying its status as the succeeding most commercially exploited in Japan, China and Korea. Often viewed as a cheaper alternative to *C. sinensis* (Li & Kan, 2017). *C. pruinosa* Petch is another species used by indigenous healers for curing intestinal conditions and inflammation-related conditions. *C. bassiana* is used for the treatment of such as dermatitis and eczema, along with as a physiological insect repellent for controlling pests (Zhao et al., 2019). In TCM, historical usage of *C. cicadae* involves treating infantile elevated temperature, convulsions and tremors.

Furthermore, medicinal attributes, like reno-protective, immunoregulatory and antitumor effects, are linked with this species (Kushwaha et al., 2020). Similarly, *C. gunnii* (Berk.) demonstrates immunomodulatory activity, enhances memory, and retards senescence. *C. guangdongensis* is used to alleviate weakness, oxidative stress, avian influenza, inflammation, and renal failure (Das et al., 2021). Conversely, *C. ophioglossoides* has historical utility as food, displaying anti-aging, antitumor, and estrogenic properties. It has also been utilized during childbirth to prevent excessive bleeding in women (Chen et al., 2020). Since the beginning of the 2000s, the conventional application of *Cordyceps* spp. has grown into an herbal remedy that is receiving a lot of focus from the promotion community. Because of its numerous health advantages, it is taken as a supplement to the diet in many different countries.

Cordyceps spp. holds a significant place in ethnopharmacy, having been used for centuries in conventional medicine across various cultures, particularly in Asian societies. In traditional Chinese medicine, *Cordyceps sinensis* and other species within the genus have been esteemed for their diverse medicinal properties. The applications of *Cordyceps* in traditional practices encompass a wide range of health benefits, including its use as an immune system booster, energy enhancer, and overall vitality tonic. Folk remedies often involve *Cordyceps* formulations to address respiratory ailments, kidney disorders, and fatigue. Its adaptogenic qualities are recognized in traditional healing systems, where it is believed to help the body adapt to stressors and restore equilibrium. Furthermore,

Cordyceps has been historically employed to support respiratory health, addressing conditions such as coughs and bronchitis. The rich cultural and historical context of *Cordyceps* spp. in ethnopharmacy underscores its enduring significance as a natural remedy with a wide array of traditional uses.

There has been a noteworthy advancement in the recent years on the availability of *Cordyceps* in the Eastern Himalayan region, predominantly on their ecology, diversity and pharmacology. Nevertheless, Further study needs to be done to fill the gap.

Most of the species of *Cordyceps* reported in the Eastern Himalayan Region of India is data deficient in terms of taxonomy, diversity, host interaction and distribution. A thorough study on their taxonomy and preferential host and also their ecological preferences is indispensable. Thus, a detailed study of its morphological characteristics which includes micromorphological and macromorphological studies along with modern taxonomic approach like molecular taxonomy becomes significant for their proper scientific identification and therefore any studies thereafter, which are dependent on correct identification. A comprehensive survey is necessary to assess the species diversity, its distribution, host specificity and habitat preferences of the region.

Cordyceps have been extensively studied for their pharmaceutical and biotechnological application. Inadequate literature is available on its bioactive components of the Cordyceps prevalent in the region. Thus, investigating of its composition and therapeutical application could lead to exploration of novel compounds with wide application in healthcare, agriculture and biological application. Along with its therapeutic, ecological and economic importance, its research for conservation and sustainable management is also extremely essential.

Addressing these research gaps will not only enrich the understanding of *Cordyceps* species in the Eastern Himalayan region but also tremendously contribute to biodiversity conservation, sustainable resource management, and the discovery of novel bioactive compounds with potential biomedical applications. Collaborative efforts involving taxonomists, ecologists, pharmacologists, and local communities are essential to address these research needs effectively. Hence, the following objectives are taken for the study

Objectives

a. To conduct thorough survey of the select areas of Eastern Himalayan region of India for detailed study of candidate *Cordyceps* spp. and its micro-habitat

b. To study the identification of the candidate *Cordyceps* spp*.,* nutritional status and genetic diversity by following standard protocols

c. To study the antioxidant and anti-microbial activity of *Cordyceps* spp.

d. To study the *in-vitro* response of bioactive components of candidate *Cordyceps* spp.in different cell lines.