Chapter: 1

Introduction
1. Introduction

Though there is availability of variety of approaches for the discovery of therapeutics but natural products are still preferred over others as they have no side effects. It has been found that about 50% of prescription drugs sold in the United States are from either natural products or its structural modified products (Lam, 2007; Ji et al., 2009). Due to this, study of chemistry of natural products is gaining importance day by day. Still there are limited studies to investigate the phyto-chemistry and pharmacological activity of medicinal herbs. Today, the research facilities have enabled researchers to carry out production, separation of bioactive molecules. There are modern tools such as ultraviolet, infrared, nuclear magnetic resonance and mass spectrometry, which can help to produce and identify the individual compound in a very short period of time.

Medicinal mushrooms have been known for thousands of years to produce biometabolites which are used or studied as possible treatment for diseases. Over two-third of cancer-related deaths could be prevented or reduced by modifying our diet with mushrooms, as they contain antioxidants (Borchers et al., 2004; Zaidman et al., 2005). *Cordyceps militaris* is also one of the medicinally important mushrooms, which has remarkable pharmacological activity and still require a lot of research to make it available for mankind. Genus *Cordyceps* have a history of medicinal use spanning millennia in parts of Asia (Gu et al., 2007). The name *Cordyceps* has been derived from two Latin words, i.e., cord and ceps meaning club and head, respectively. *Cordyceps militaris* belongs to the Phylum *Ascomycota* classified in the Order *Hypocreales*, as spores are produced internally inside a sac, called ascus (Wang et al., 2008). It is an entomopathogenic fungus having an annual appearance which often grows parasitically on lepidopteron larvae and pupae of insects and spiders. It normally inhabits on the surface of insects pupae in winters and leading to the formation of fruiting body in summers justifying its name as “winter-worm summer-grass”.
*Cordyceps* has been included as one of the growing number of Fungal Traditional Chinese Medicine (FTCM) used as cures for modern diseases with many products available commercially. Due to recent advancements in pharmaceutical bio-techniques, it is possible to isolate bioactive compounds from *Cordyceps* and make it available in powder as well as in capsular form (e.g. Didanosine). Past five years have seen tremendous exploitation of *Cordyceps* which significantly reduced its wild occurrence (Negi, 2006; Winkler, 2008). Efforts have been made to artificially cultivate this mushroom by surface and submerged fermentation techniques. The ancient medicinal mushroom *C. militaris*, which has been used as a crude drug for the welfare of mankind in old civilization, is now a matter of extensive research to identify the individual compound responsible for pharmacological activity.

*Cordyceps*, especially its extract contains many biologically active compounds including Cordycepin, cordycepic acid, adenosine, exo-polysaccharides, vitamins and enzymes. Out of these, Cordycepin or 3′-deoxyadenosine (9-(3-deoxy-β-D-ribofuranosyl) adenine) is the main active constituent which is most widely studied for its medicinal value having a broad spectrum biological activity (Cunningham, 1950). The structure of Cordycepin is very much similar with cellular nucleoside, adenosine and acts like a nucleoside analogue. The molecular formula and molecular weight for cordycepin is C$_{10}$H$_{13}$N$_{5}$O$_{3}$ and 251.24 respectively. Cordycepin is an alkaline, needle-like or flaky crystal, melting point 228°C–231°C, with a maximum absorption wavelength of 259.0 nm.

Cordycepin is known to interfere with various biochemical and molecular processes including purine biosynthesis (Overgaard, 1964; Rottman and Guarino, 1964), DNA/RNA synthesis (Holbein et al., 2009) and mTOR (Mammalian Target of Rapamycin) signaling transduction (Wong et al., 2010). In the western world, lung cancer is still leading cause of mortality and responsible to claim 170,000 deaths per year which is higher than the sum of deaths due to breast, colon, and prostate cancer (Danesi et al., 2003). Approximately 80–85%
of all lung cancers are classified as non-small-cell lung cancer (NSCLC). It is very hard to cure NSCLC even in early stage as it develops resistance against radiation and chemotherapy. Due to this, cure of NSCLC remains elusive despite the availability of a variety of chemotherapeutics agents that exhibit sophisticated mechanism of action (Kim et al., 2003a; Jemal et al., 2010).

Recently targeting apoptosis, also referred to as programmed cell death, comprises an important mechanism in many anti-cancer drugs, including cordycepin. Second approach is to target angiogenesis which is a complex and tightly controlled physiological process by which new blood vessels are originated from pre-existing capillaries (Folkman, 1995; Risau, 1997). Tumor cells can induce angiogenesis through the activation of endothelial cells followed by pro-angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF) (Jain, 2002; Kaya et al., 2000; Weidner et al., 1991). These factors are highly expressed and associated with growth of various types of human tumors (Ferrara et al., 2003; Breier and Risau, 1996). In the last few decades, a large number of natural bioactive metabolites including paclitaxel, vinblastine and camptothecin have been isolated and used to treat cancers. The inadequate availability of cancer treatment invites scientific communities to propose the development of novel therapeutic strategies for cancer treatment. Therefore, compounds with apoptotic and anti-angiogenic properties have been identified as an attractive strategy for the treatment and prevention of cancer.

The present study was carried out to fill this vacuum and focusing on efficient production and extraction of active content of Cordyceps militaris (cordycepin) followed by evaluation of its anti-proliferatory and anti-angiogenic activity. Taking into account of these issues, the following objectives were set and tried best to achieve them.
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Objectives

1. Isolation and optimization of cordycepin extraction from *Cordyceps militaris*.
2. Biochemical analysis of isolated cordycepin to know its purity.
3. Evaluation of anti-cancerous activity of cordycepin on adherent cancer cell lines.