ABSTRACT

Eastern Himalayan region is a biodiversity hot spot. The region is dominated with a temperate broadleaf forest ecoregion found in the middle elevations of the Eastern Himalayas, including parts of Nepal, India, Bhutan, Myanmar and China. These forests have an outstanding richness of high diversity of flora and fauna. Mycophagy is a common practice in Eastern Himalayan region. Entomopathogenic fungi *Cordyceps* spp. is a genus of parasitic fungi. They exhibit immunomodulatory, anti-inflammatory, antioxidant, and anti-tumor properties. Nucleotides Cordycepin (3′-deoxyadenosine) and adenosine exhibit immunomodulatory and antioxidant activities. Fungi contains sterols, including ergosterol, which plays a crucial role in vitamin D2 synthesis. Cordycepin from *Cordyceps* spp. has shown potential in restraining inflammatory mediators in neurodegenerative diseases. With these consideration and background, the present study was carried out to collect and identify *Cordyceps* spp. through morphological, microscopic and phylogenetic analysis from select areas of Eastern Himalayan belt. Morphological studies (macro and micromorphological) were carried out and fresh sample collected were cultured. The DNA was isolated and PCR amplification was carried out using *ITS*, *COI* primer. Sequencing was carried out using sanger dideoxy method and it was processed in BioEdit version 7.2.5. Nucleotide BLAST tool in NCBI database was used to evaluate the homology and similarity with existing database of the collected sample. Subsequently, phylogenetic tree was constructed distinctly for *ITS* and *COI* amplicons. Phylogenetic study of the *ITS* gene along with microscopic characteristics confirmed that CBUS1-CBUS4 (Cordyceps Bodoland University Sikkim) collected from Sikkim to be *Ophiocordyceps sinensis*. Similarly, phylogenetic study of *COI* gene of CBUS1-CBUS4 confirmed the larva to be of *Thitarodes* spp. CBUAP1 (Cordyceps Bodoland University Arunachal Pradesh 1) collected from Mechuka valley was identified as *Ophiocordyceps liangshanensis* (first report from India). The sample collected from Bhutan CBUB1-B4 (Cordyceps Bodoland University Bhutan) was identified as *Ophiocordyceps sinensis*, with the host larva *Thitarodes* spp. The protein content of all the samples were investigated. CBUS4 (*Ophiocordyceps sinensis*) exhibited the highest protein content of 14.1% (±0.01), whereas CBUCM (*Cordyceps militaris*) grown on Brown Rice exhibited the lowest protein content of 5.25% (±0.02). Total Dietary fiber of the samples was evaluated, CBUS3 (*Ophiocordyceps sinensis*) demonstrated the highest total dietary fiber content of 42.72% (±0.05), CBUS3- *Mycelia* (*Ophiocordyceps sinensis*) exhibited the lowest total dietary fiber content of 21.33(±0.08). Among the *Cordyceps militaris* samples cultivated on

different rice varieties, brown rice exhibited the highest fiber content of 34.25% (±0.03). *In vitro* free radical scavenging activity was studied of all the samples following DPPH assay, FRAP assay and $ABTS^{+}$ cation scavenging assay. From the result of antioxidant activity, the sample CBUS3 was found to possess the highest overall antioxidative potential. The methanolic aqueous extract (70:30) of the samples was evaluated for its antimicrobial properties against six bacterial strains, the samples exhibited with variable intensity. CBUB2 and *Cordyceps militaris* grown on Joha rice exhibited the highest inhibition against *Staphylococcus aureus* with 27 mm zone of inhibition. Similarly, CBUS3 exhibited the highest inhibition against *Salmonella typhi* and *Mycobacterium smegmatis* with 20 mm and 19 mm zone of inhibition respectively. *Cordyceps militaris* grown on Basmati rice recorded the highest inhibition of *Pseudomonas aeruginosa* with 18 mm zone of inhibition. CBUS3 exhibited highest inhibition of 14 mm against *Escherrichia coli*. Similarly, CBUS3 exhibited highest inhibition of 14 mm against *Bacillus cereus*. *In vitro* cytotoxicity of extracts prepared from the fruiting bodies and mycelia of CBUS3 (*Ophiocordyceps sinensis*) and CBUAP1 (*Ophiocordyceps liangshanensis*) was studied in the MCF-7, HeLa, and SKOV3 cell lines. CBUS3 (mycelia) exhibited potent activity with an IC_{50} 77.48. The cytotoxicity assessment determined by the release of LDH for samples CBUAP1, CBUS3 (fruiting), and CBUS3 (mycelia) against MCF-7 cells was significantly increased at concentrations of 1891.54 U/L, 1554.91 U/L, and 1944.97 U/L, respectively, at a dose of 320 µg/ml. Similarly, the LDH release against the HeLa cell line was increased at a concentration of 1656.43 U/L, 1496.13 U/L, and 1854.14 U/L, and against the SKOV3 cell line at a concentration of 1683.15 U/L, 1474.76 U/L, and 1907.57 U/L at a dose of 320 µg/ml. High Performance Liquid Chromatography was used for the quantification of adenosine and cordycepin. The results demonstrated that the wild collected samples CBUS3 had the highest adenosine content of 119 µg/ mg of extract. Cordycepin was found only from CBUAP1 at a concentration of 10 µg/ mg. *Cordyceps militaris* grown on barni rice had the highest concentration of adenosine content of 260 µg/mg extract and the lowest concentration of adenosine was found to be on *Cordyceps militaris* grown on basmati rice 145 µg/mg. The concentration of cordycepin was found to be highest in *Cordyceps militaris* grown on brown rice of 300µg/ mg, and the lowest concentration of cordycepin was recorded from *Cordyceps militaris* grown on black rice 100 µg/mg of extract. The samples were analysed using GCMS, which allowed for the identification of several bioactive compounds based on their peaks observed on a chromatogram. The peaks were subsequently compared to the peaks of recognised compounds recorded in NIST libraries. The fragmentation of molecules was compared based

on their mass-to-charge ratio (m/z), and the compounds were identified. GCMS analysis revealed the identification of potent pharmacologically important bioactive compounds like clofexamide, cyclobarbital, succinic acid, [1,4] dioxino[2,3-b]-1,4-dioxin, hexahydro-2,2,3,3-tetramethyl and flecainide which has been reported to be antidepressant, antimicrobial, cytotoxic activities and antiarrhythmic activity (used for treatment of irregular heartbeat). The current study concludes that the samples collected from Sikkim to be *Ophiocordyceps sinensis*, proper scientific identification was absent previously. The second major finding in our research is the first report of *Ophiocordyceps liangshanensis* from India, the sample was collected from Tsi-yomi district of Arunachal Pradesh. The antioxidant and antimicrobial study suggested wild *Ophiocordyceps sinensis* had better activity in comparison with standard *Cordyceps militaris* strain. The major bioactive compounds cordycepin and adenosine was found to be higher in standard *Cordyceps militaris* strain in comparison with the wild samples (*Ophiocordyceps sinensis* & *Ophiocordyceps liangshanensis*). The finding of GCMS analysis suggested presence of potent anti-depressant compounds along with anti-arrhythmic and compounds used in the treatment of respiratory infections.