Anti-Inflammatory, Antimicrobial and Cytotoxic Properties of Cordyceps Militaris, a Native Indian Medicinal Fungus, Against KB Cells

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**Abstract:** Cordyceps militaris, a medicinal fungus native to India, is recognized for its bioactive properties, including antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities. This study evaluated the therapeutic potential of C. militarisextracts through various assays. Antioxidant activity was measured using the DPPH assay, demonstrating dose-dependent efficacy comparable to Vitamin C at 500 µg/ml. Anti-inflammatory properties were assessed via albumin denaturation inhibition, while cytotoxicity against KB cancer cells was evaluated using the MTT assay, revealing an IC50 value of 125 µg/ml. UV-Vis and FTIR spectroscopy identified bioactive functional groups in the extract, while antimicrobial activity was tested under anaerobic conditions, confirming its efficacy against anaerobic microbes. Statistical analysis (p < 0.05) validated the results. The findings highlight the bioactive potential of C. militaris, particularly its therapeutic applications in combating oxidative stress, inflammation, microbial infections, and cancer, underscoring its potential as a valuable resource in pharmacology and oncology.

**Keywords:** *Cordyceps militaris*; antioxidant, anti- inflammatory, antimicrobial, cytotoxicity

# INTRODUCTION

Medicinal plants have been used as therapeutic agents since ancient times, and more than 10,000 plants are used for medicinal purposes [(Rupa et al., 2020)](https://paperpile.com/c/fo0Kqx/n1hR). Cordyceps militaris (C. militaris) has recently gained significant attention due to its novel bioactive properties, making it a promising candidate for pharmaceutical and therapeutic applications[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/fo0Kqx/6rOu+sbT9+K2TB). It is the largest and most diverse genus classified in the family Clavicipitaceae as regards to the number of its species, the morphology and the variation in its host, with over 750 identified species [(Olatunji et al., 2018)](https://paperpile.com/c/fo0Kqx/5TBv). Traditional medicine recommends the use of Cordyceps spp. for treating several human disorders such as cardiovascular and respiratory diseases, disorders of the liver, cancers, diabetes, infectious and parasitic diseases and sexual dysfunctions [(Jędrejko et al., 2021)](https://paperpile.com/c/fo0Kqx/mF3g). Cordyceps militaris (C. militaris) has been used in Eastern countries for the treatment of various diseases including chronic kidney diseases [(Gu et al., 2013)](https://paperpile.com/c/fo0Kqx/n9X1). Many bioactive molecules of nutraceutical interest, such as cordycepin(3’-deoxyadenosine), ergosterol, trehalose, mannitol, and several polysaccharides, nucleosides, and amino acids have been detected in or isolated from C. militaris, and these are thought to have potential antiaging, whitening, antitumor, anti-inflammatory, immunomodulatory, and blood glucose and cholesterol-lowering activities [(Zhang et al., 2023)](https://paperpile.com/c/fo0Kqx/xfkp).

Cordycepin (3'-deoxyadenosine) an adenosine analog, is one of the major bioactive components of C. militaris, and has a number of pharmacological properties [(Ganapathy et al., 2021)](https://paperpile.com/c/fo0Kqx/bYu6). It was found that 3’-deoxyadenosine suppressed expression of collagens induced by Transforming growth factor-beta 1 (TGF Beta 1) and may be useful for therapeutic intervention in various TGF-beta-related fibrotic disorders [(Gu et al., 2013)](https://paperpile.com/c/fo0Kqx/n9X1). A polysaccharide from the aqueous extract of cultured C. militaris was found to demonstrate anti-inflammatory activity [(Ng & Wang, 2005)](https://paperpile.com/c/fo0Kqx/CcM7). The 70% ethanolic extracts of cultured fruiting bodies and mycelia of C. militaris applied topically exhibited anti-inflammatory effect in the croton oil-induced ear oedema test in mice [(Ng & Wang, 2005)](https://paperpile.com/c/fo0Kqx/CcM7). Cordycepin and related compounds may also provide another avenue for the discovery of clinically useful antifungal drugs [(Sugar & McCaffrey, 1998)](https://paperpile.com/c/fo0Kqx/JboN). C. militaris showed active functions especially in the treatment of respiratory, liver, renal dysfunction, heart and lung diseases, hyperglycemia, hyperlipidemia, and as antitumor/anticancer agents [(Olatunji et al., 2018)](https://paperpile.com/c/fo0Kqx/5TBv). As per studies, the exploration of cordycepin-induced cell death would be valuable to design more effective chemotherapy agents against Head and Neck squamous cell carcinoma [(Sugar & McCaffrey, 1998)](https://paperpile.com/c/fo0Kqx/JboN). The aim of this study was to assess antimicrobial, antioxidant, and cytotoxic effects of C. militaris to determine their therapeutic potential[(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/fo0Kqx/qQmF+r6f4)(Chokkattu et al. 2022; Ramamurthy et al. 2022). By assessing these biological activities, the research seeks to understand the bioactive compounds responsible for these effects and their mechanisms of action[(Marya et al., 2022)](https://paperpile.com/c/fo0Kqx/OQ8ja) [(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/fo0Kqx/OQ8ja+2sBEd) [(Wadhwani et al., 2022)](https://paperpile.com/c/fo0Kqx/8UmCa). The study also aims to explore the potential application of Cordyceps militaris in developing novel therapeutic interventions for managing oxidative stress, inflammation, and microbial infections. This investigation provides a foundation for further exploration of C militaris as a natural and sustainable source for medical and pharmaceutical advancements.

# MATERIALS AND METHODS

## Chemical and reagents

The study utilized various chemicals and reagents, including Cordyceps militaris powder procured from Himadri Biotechnology, Jodhpur) for extraction using analytical-grade ethanol in a Soxhlet apparatus. For antioxidant assays, DPPH, ABTS, and pyrogallol were employed, with ascorbic acid serving as the standard. Anti-inflammatory activity was assessed using egg albumin, phosphate-buffered saline (PBS), and positive controls like ibuprofen and diclofenac. For the MTT cell viability assay, MTT and dimethyl sulfoxide (DMSO) were used alongside Dulbecco’s Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) and penicillin-streptomycin solution. Additionally, 1X PBS was used for cell washing, and MCF-7 breast cancer cells (from NCCS, Pune) served as the test cell line.

## Extraction and characterization of *Cordyceps militaris*

Extraction of *Cordyceps militaris* was achieved by treatment of 100g of the mushroom procured from Himadri Biotechnology, Jodhpur. The dried *Cordyceps militaris* mushrooms were ground into a fine powder and subjected to different concentrations of ethanol extraction using a Soxhlet apparatus for 8 hours. The extract was concentrated using a rotary evaporator under reduced pressure to remove the solvent. The resulting crude extract was stored at 4°C for further analysis.

## Bioactivity of Cordyceps militaris

## Total Phenolic Content

The Total Phenolic Content (TPC) was determined using the Folin–Ciocalteu assay. For the analysis, 0.25 mL of the crude ethanolic extract (1 mg/mL) was mixed with 1 mL of Folin–Ciocalteu’s reagent, which had been diluted 10-fold with water. The mixture was incubated in the dark for 5 minutes, followed by the addition of 2 mL of sodium carbonate solution (7.5% w/v). The optical density was measured at 765 nm using a spectrophotometer. The results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract) using a standard curve prepared with gallic acid as the reference compound [(Das et al., 2024; Molole et al., 2022)](https://paperpile.com/c/fo0Kqx/V7My+ackE).

## DPPH radical scavenging activity

The radical scavenging activity of the ethanolic extract of *Cordyceps militaris* against the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured spectrophotometrically. To assess the DPPH scavenging activity, a DPPH solution was prepared by dissolving 12.5 mg of DPPH in 50 mL of ethanol. An aliquot of 100 μL of the ethanolic crude extract (100 μg/mL) was added to 1 mL of the DPPH solution. The reaction mixture was incubated at 37°C in the dark for 20–30 minutes. The decrease in absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as the standard, and the experiment was conducted in triplicate. The scavenging activity was calculated using the following formula:

Q (%)= Absorbance of control- Absorbance of sample/ Absorbance of control X 100

where Q represents the DPPH scavenging activity [(Baliyan et al., 2022)](https://paperpile.com/c/fo0Kqx/VrcQ).

## Total antioxidant activity by ABTS Assay

The ABTS assay was used to evaluate the total antioxidant activity (TAA) of the extract. Stock solutions of ABTS (7.4 mM) and potassium persulfate (2.46 mM) were prepared and mixed in equal volumes to generate the ABTS∙⁺ radical solution. The mixture was allowed to react for 12 hours at room temperature in the dark. The working solution was prepared by diluting 1 mL of the ABTS∙⁺ solution with 60 mL of methanol. Fresh ABTS∙⁺ solution was prepared for each assay. For the assay, 150 μL of the crude extract was added to 2850 μLof the ABTS∙⁺ solution. The reaction mixture was incubated in the dark for 2 hours, after which the absorbance was measured at 734 nm using a spectrophotometer. The total antioxidant activity was expressed as ascorbic acid equivalents (mg AAE/g extract), determined using a standard curve prepared with ascorbic acid as the reference compound [(Nithin Krishna et al., 2024)](https://paperpile.com/c/fo0Kqx/PonK).

## Estimation of reducing power

1 mL of the extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (w/v) potassium hexacyanoferrate solution. The mixture was incubated at 50°C for 30 minutes, followed by the addition of 2.5 mL of 10% (w/v) aqueous trichloroacetic acid (TCA). After centrifugation for 10 minutes, 2.5 mL of the supernatant was mixed with 2.5 mL of water and 0.5 mL of 0.1% FeCl₃ solution. The absorbance was measured at 700 nm using a spectrophotometer. The results were expressed as ascorbic acid equivalents (mg AAE/g extract), with ascorbic acid serving as the positive control [(Das et al., 2024; Irshad et al., 2012)](https://paperpile.com/c/fo0Kqx/pMKp+ackE).

## Anti-inflammatory activity albumin denaturation inhibition

The Albumin denaturation inhibition assay as described by Praveen et al. 2024, was followed. Positive standards, ibuprofen and diclofenac were prepared as 0.1 percent each, that is 1.0 mg/ml with plat drug*.* Each reaction vessel contained 1000 µl of the test compound, 1400 µl of PBS and 200 µl of egg albumin. In place of the compound, distilled water was used as a negative control. After 15 minutes of incubation at 37 °C, the mixtures were heated for 5 minutes at 70 °C. Absorbances at 660 nm were measured after cooling. The percentage of protein denaturation inhibition was calculated according to the formula given below:

Inhibition %= (Absorbance of Control-Absorbance of Test Sample)/(Absorbance of Control)×100 (Praveen et al. 2024)

## Determination of cell viability by MTT assay for the cells treated by extracts of *Militaries*

Cell culture: KB cell lines were obtained from NCCS, Pune, India. The cells were regularly passaged and cultured in a CO2 incubator, using a culture medium consisting of 10% fetal bovine serum (FBS), a mixture of 1% penicillin and streptomycin antibiotics, and DMEM media from Himedia.Cell viability was assessed using MTT. KB cells (1x105 cells/ml) were cultured for 24h & 48h on 96-well microplates. The cells were incubated for different time-points for different modes of studies with and without drugs [15]. The materials required for MTT included, freshly prepared MTT (5mg/ml in serum-free media) kept in the dark at 4ºC, DMSO, 96 flat bottom well plates were used and DMEM (Complete media/Serum free media) along with 1XPBS was also used [(Jayaraman et al., 2024)](https://paperpile.com/c/fo0Kqx/wFOu). Cells (1x105) were plated in a 96-well plate supplemented with ~100μl cell culture media in each well. After treatment was over, 10μl of MTT solution was added to each well and mixed properly. Cells were then incubated for 4h in the dark at 37ºC. After that incubation period, the media from each well was removed out and formazan crystals were dissolved with 100μl DMSO to each well followed by mixing them well. Then the mixture was incubated again, in the dark at 37ºC for 30 Minutes. Finally, the absorbance of the dissolved formazan reagent was measured by using a microplate reader at 595nm [(Jayaraman et al., 2024)](https://paperpile.com/c/fo0Kqx/wFOu).

Calculation was done using the given formula:

Viable cells (%) = OD of drug treated sample/ OD of control sample x 100

## Functional characterization of extract of *C. militaris* through FTIR

The *C. militaris* mushroom samples were collected, cleaned thoroughly, and dried to remove moisture content. The dried samples were then ground into a fine powder to ensure uniformity for analysis. The powdered sample was prepared for Fourier Transform Infrared (FTIR) spectroscopy using a standard protocol designed to achieve optimal spectral results. The prepared sample was analyzed using an FTIR spectrometer in the mid-infrared region (4000–500 cm⁻¹). A background spectrum was first recorded to eliminate interference from atmospheric gases or the instrument. The FTIR spectrum of the sample was then obtained, and the resulting absorption peaks were analyzed to identify functional groups such as amino acids, fatty acids, aliphatic groups, carboxylic acids, and alcohols, providing insights into the chemical composition of *C. militaris*.

## Statistical analysis

Data was expressed by the means ± SEM of three independent experiments, each conducted in triplicate. Statistical analysis will be carried out using one-way ANOVA, with *p<0.05* indicating statistical significance.

# Results and Discussion

## Total Phenolic Content of *C. militaris* extract

The Total Phenolic Content (TPC) of the ethanolic extract (EtE) of *C. militaris* was determined to be 174.09 mg GAE/g extract, indicating its substantial phenolic composition. This result underscores the efficiency of ethanol as a solvent for extracting phenolic compounds, which are known for their antioxidant properties. The TPC value suggests that the ethanolic extract holds significant potential for applications where phenolic content is a key factor.

## Antioxidant assay using DPPH assay

The radical scavenging activity of the ethanolic extract of *C. militaris* was assessed using the DPPH assay, with ascorbic acid as the standard. The IC50 value, which indicates the concentration required to scavenge 50% of DPPH radicals, was 70.10 ± 3.76 μg/mL for the ethanolic extract. In comparison, the standard ascorbic acid showed a lower IC50 value of 52.91 ± 3.87 μg/mL, reflecting its superior radical scavenging efficiency. While the ethanolic extract demonstrated significant antioxidant activity, its scavenging ability was slightly lower than that of the standard. These results confirm the ethanolic extract's potential as a natural antioxidant.

## Total antioxidant activity

The total antioxidant activity (TAA) of the ethanol extract of *C. militaris* was determined using the ABTS assay, with results expressed as ascorbic acid equivalents (mg AAE/g extract). The extract exhibited a significant antioxidant potential, with a TAA value of 862.79 mg AAE/g extract. The IC50 value, which represents the concentration required to scavenge 50% of the ABTS radicals, was found to be 50.30 μg/mL for the ethanol extract. In comparison, the standard antioxidant, ascorbic acid, displayed a lower IC50 value of 48.42 μg/mL, indicating slightly superior antioxidant activity. These findings suggest that the ethanol extract has substantial antioxidant properties, comparable to ascorbic acid, and could serve as a potential natural antioxidant source (Barapatre et al. 2016).

## Estimation of reducing power

The reducing power of the ethanolic extract of *C. militaris* was assessed using the method involving potassium hexacyanoferrate and trichloroacetic acid. The extract demonstrated a notable reducing power of 403.89 mg AAE/g extract, indicating its significant ability to reduce metal ions and suggesting its potential antioxidant capacity. Ascorbic acid was used as a positive control in this assay, providing a benchmark for comparison. These results highlight the C. militaris ethanolic extract's potential as a source of natural reducing agents [(Aadil et al., 2014)](https://paperpile.com/c/fo0Kqx/RpH4).

## Anti-inflammatory activity of ethanolic extract of *C. militaris*

Anti-inflammatory property of extract by protein denaturation assay. The anti-inflammatory property for extract was examined by protein denaturation assay compared with standard (Ibuprofen). The result showed a significant result of increased activity in increasing concentration varying from 100-500 μg/ml.

The peak- anti-inflammatory efficacy, for both the extract and the standard agent, materialized at the concentration of 300&500 μg/ml, as illustrated in Figure 2. Statistical significance was determined at p < 0.05. Differences between groups were considered significant when indicated by (\*\*) for p < 0.01 and (\*\*\*) for p < 0.001.

## Cell- Viability assay

The results of the MTT assay for KB cells are presented in the Figure 3, showing the effect of different concentrations of the ethanolic extract of *C. militaris* (ranging from 0 to 350 μg/mL) on cell viability. The viability of cells decreases in a concentration-dependent manner. At 50 μg/mL, cell viability was estimated to be 80%, indicating a mild inhibitory effect. As the concentration was increased to 100 μg/mL, cell viability decreased to around 60%. A further increase in concentration to 150 μg/mL reduced cell viability to about 50%. At higher concentrations, such as 200–350 μg/mL, a significant decline in cell viability was observed, with values reaching approximately 30–40%, suggesting a strong cytotoxic effect at these doses. As shown in Figure 3, cells treated at 7 different concentrations (50-350 microgram/ml), the inhibitory concentration was found to be 125 microgram further the optimal dose (125 μg) was further evaluated for cytotoxicity. These results showed that the IC₅₀ concentration did not show any cytotoxicity. This concentration-dependent decrease in cell viability indicates the potential cytotoxic nature of the extract of *C. militaris*, which becomes more pronounced at higher concentrations.

## Functional characterization of extract of *C. militaris*

The FTIR analysis of *C. militaris* revealed several significant functional groups contributing to its chemical properties and bioactivity. The broad absorption band at approximately 3259.79 cm⁻¹ corresponds to H-N-H stretching, indicating the presence of amino groups, which are associated with proteins or amino acids in the sample. The peak at 2928 cm⁻¹ represents C-H stretching from aliphatic groups, suggesting the presence of fatty acids or hydrocarbons. A distinct band at 1626.72 cm⁻¹ corresponds to C=C stretching, indicative of unsaturated fatty acids, which may contribute to antioxidant or anti-inflammatory properties(Rafi et al., 2024). The peak at 1238.10 cm⁻¹ corresponds to C-O stretching of carboxylic acids, while the band at 1082.58 cm⁻¹ is associated with C-O stretching in primary alcohols. These functional groups collectively highlight the bioactive potential of *Cordyceps militaris*, supporting its use in therapeutic and nutraceutical applications (Tuluwengjiang et al., 2024).

The FTIR spectrum displayed the transmittance of functional groups in the sample, spanning a wavenumber range of 4000–500 cm⁻¹ (Figure 4).

• 3259.79 cm⁻¹ corresponding to H-N-H stretching (amino group),

• 2928 cm⁻¹ for C-H stretching (aliphatic),

• 1626.72 cm⁻¹ for C=C stretching (unsaturated fatty acid),

• 1238.10 cm⁻¹ for C-O (carboxylic acid), and

• 1082.58 cm⁻¹ for C-O (primary alcohol).

The present study assessed the antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties of *Cordyceps militaris*, a novel medicinal mushroom originating from India and the Himalayan region [(Chokkattu et al., 2023)](https://paperpile.com/c/fo0Kqx/AG44a)[(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/fo0Kqx/AiZ9+4qOs)[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/fo0Kqx/sjBqA) . This mushroom had gained increasing attention for its unique therapeutic properties. We assessed the properties of Cordyceps militaris through DPPH, albumin denaturation, and MTT assays. As shown in Figure 5, C.militaris dose dependently increased the DPPH radical formation from the concentration of 100-500 microgram/ml concentration while 400 & 500 microgram/ml doses showed the maximum free radical scavenging effect who’s efficacy was found to be close to that of the standard drug. It also exhibited strong inflammation inhibition comparable to standard drugs[(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/fo0Kqx/sjBqA+AiZ9)[(Merchant et al., 2022)](https://paperpile.com/c/fo0Kqx/r6f4)[(Sreevarun et al., 2023)](https://paperpile.com/c/fo0Kqx/JDlwN). The cytotoxicity analysis revealed an IC₅₀ value of 125 µg/ml, demonstrating its non-toxic nature and potential for therapeutic applications. The UV-Vis analysis, in the current study, revealed a prominent absorption peak at 239 nm, corresponding to the presence of carbonyl groups (C=O), indicating the compound’s conjugated system. FTIR analysis further confirmed functional groups such as amino, aliphatic, unsaturated fatty acids, carboxylic acids, and primary alcohols, which contributed to the bioactive nature of the sample. These findings supported the chemical composition and potential functional properties of the compound under study. Few other studies have also shown the antioxidant properties of cordycepin [(Oyaizu, 1986)](https://paperpile.com/c/fo0Kqx/QWlN) which stated that the antioxidant activity of C. militaris might be influenced by other chemical constituents present in fruiting bodies, e.g., ergothioneine, phenolic compounds, carotenoids and selenium [(Chan et al., 2015; Chen et al., 2012; Cohen et al., 2014)](https://paperpile.com/c/fo0Kqx/qtC8+lRmd+F6cz). The antioxidant potential of C. militaris in terms of inhibiting lipid peroxidation exceeded that of Cordyceps sinensis , which was another species belonging to the same genus as C. militaris. The antioxidant activity was related to the content of polysaccharides and phenolic compounds in C. militaris fruiting bodies [(Yu et al., 2006)](https://paperpile.com/c/fo0Kqx/G5B0). Rupa et al stated C militaris nano emulsion reported to be a better source of antioxidants than an aqueous extract of Cordyceps militaris fungus [(Rupa et al., 2020)](https://paperpile.com/c/fo0Kqx/n1hR).In subsequent in-vitro tests, the anti-inflammatory activity of C. militaris was confirmed to result from the inhibition of production of proinflammatory mediators [(Jędrejko et al., 2021)](https://paperpile.com/c/fo0Kqx/mF3g). As per Zhang et al, there is both in vitro and in vivo evidence that C. militaris and its active constituents prevent inflammation in several experimental models [(Zhang et al., 2023)](https://paperpile.com/c/fo0Kqx/xfkp). A polysaccharide from the aqueous extract of cultured C. militaris demonstrated anti-inflammatory activity (Yu et al. 2006). The 70% ethanolic extracts of cultured fruiting bodies and mycelia of C. militaris applied topically (0.5mg/ear) exhibit anti-inflammatory activity [(Ng & Wang, 2005)](https://paperpile.com/c/fo0Kqx/CcM7). Likewise, the investigation unveiled a similar trend in the inhibition of anti-inflammatory activity, with inhibition rates escalating in response to escalating concentrations (ranging from 100 to 500 μg/ml) of both the extract and the standard compound[(Adel et al., 2023)](https://paperpile.com/c/fo0Kqx/qPv2x)[(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/fo0Kqx/ZYcQ)[(Solanki et al., 2023)](https://paperpile.com/c/fo0Kqx/AxVcp). During the analysis of Anti inflammatory activity, global studies assessed C.militaris nano emulsion which showed a zone of inhibition by Cordyceps nano emulsion in E. coli and S. aureus cultures. T.B. Ng et al stated that Cordycepin inhibited the growth of Clostridium species [(Ng & Wang, 2005)](https://paperpile.com/c/fo0Kqx/CcM7). The observed antimicrobial activity for the present study was not very significant, differing from previous studies that demonstrated stronger efficacy.Studies conducting in vitro cytotoxicity and anti-inflammation assays suggested that C.militaris was not toxic [(Rupa et al., 2020)](https://paperpile.com/c/fo0Kqx/n1hR). A research conducted by Wu et al stated that cordycepin significantly induced cell apoptotsis in human oral squamous cancer cells [(Wu et al., 2007)](https://paperpile.com/c/fo0Kqx/6pJo). Cordycepin also induced cell death in a time and dose-dependent manner[(Ganapathy et al.,2021)](https://paperpile.com/c/fo0Kqx/oWH6).In conclusion, Cordyceps militaris demonstrated antioxidant activity and moderate anti-inflammatory properties suggesting potential health benefits. It exhibited moderate antimicrobial effects against anaerobic metronidazole-resistant strains but did not show significant activity against aerobic strains. Additionally, its cytotoxic effects indicated potential applications in cancer therapy.

## Data availability

The data will be available as requested to the corresponding author.

# Author contributions

PR: conceptualization, writing – original draft, methodology, investigation and validation. RD: writing – original draft, methodology, data analysis, data analysis, resources, validation. AM: methodology, data analysis, validation.

## Abbreviations

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl

MTT: (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)

## Legends to the Figure

Figure 1: Dried Cordyceps militaris

Figure 2: Anti inflammatory activity of Cordyceps militaris by Protein Denaturation inhibition.

Figure 3: Cytotoxic effects of Cordyceps militaris in KB Cells

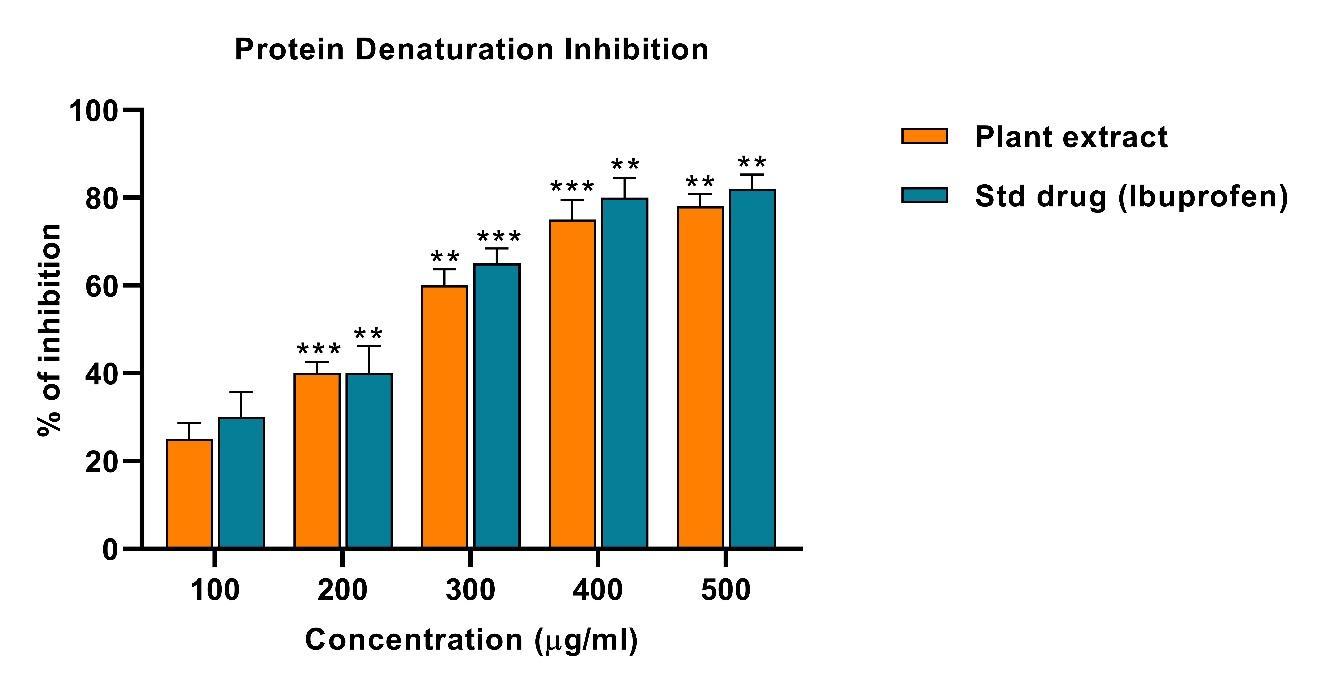
The assay was performed in triplicates. Significance was considered at the levels of p < 0.05. (\*\*) denotes significance among the groups at p<0.001; (\*\*\*) denotes p< 0.001

Figure 4: FTIR Spectrum in Cordyceps militaris

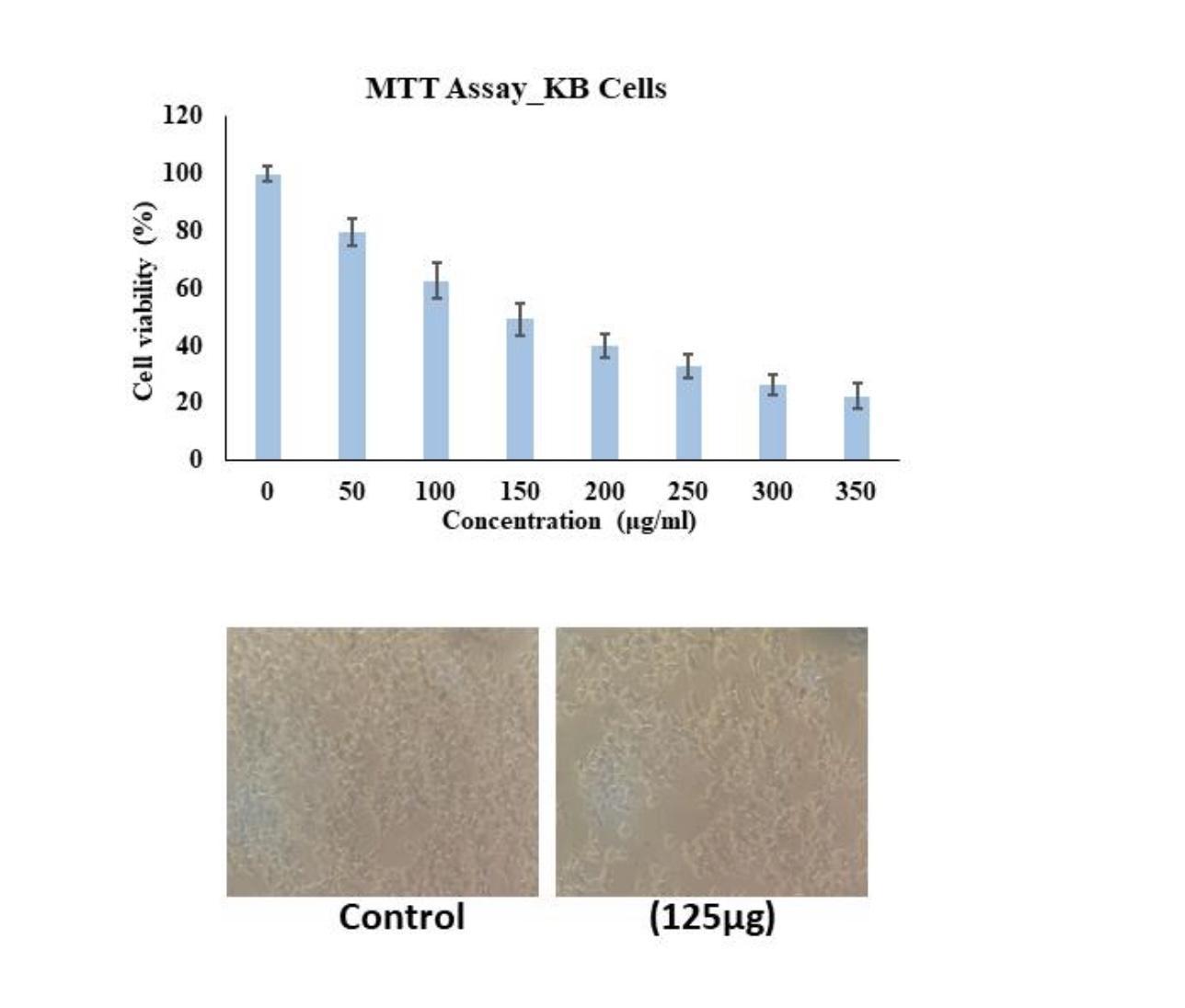
Figure 5: DPPH radical scavenging assay of Cordyceps militaris



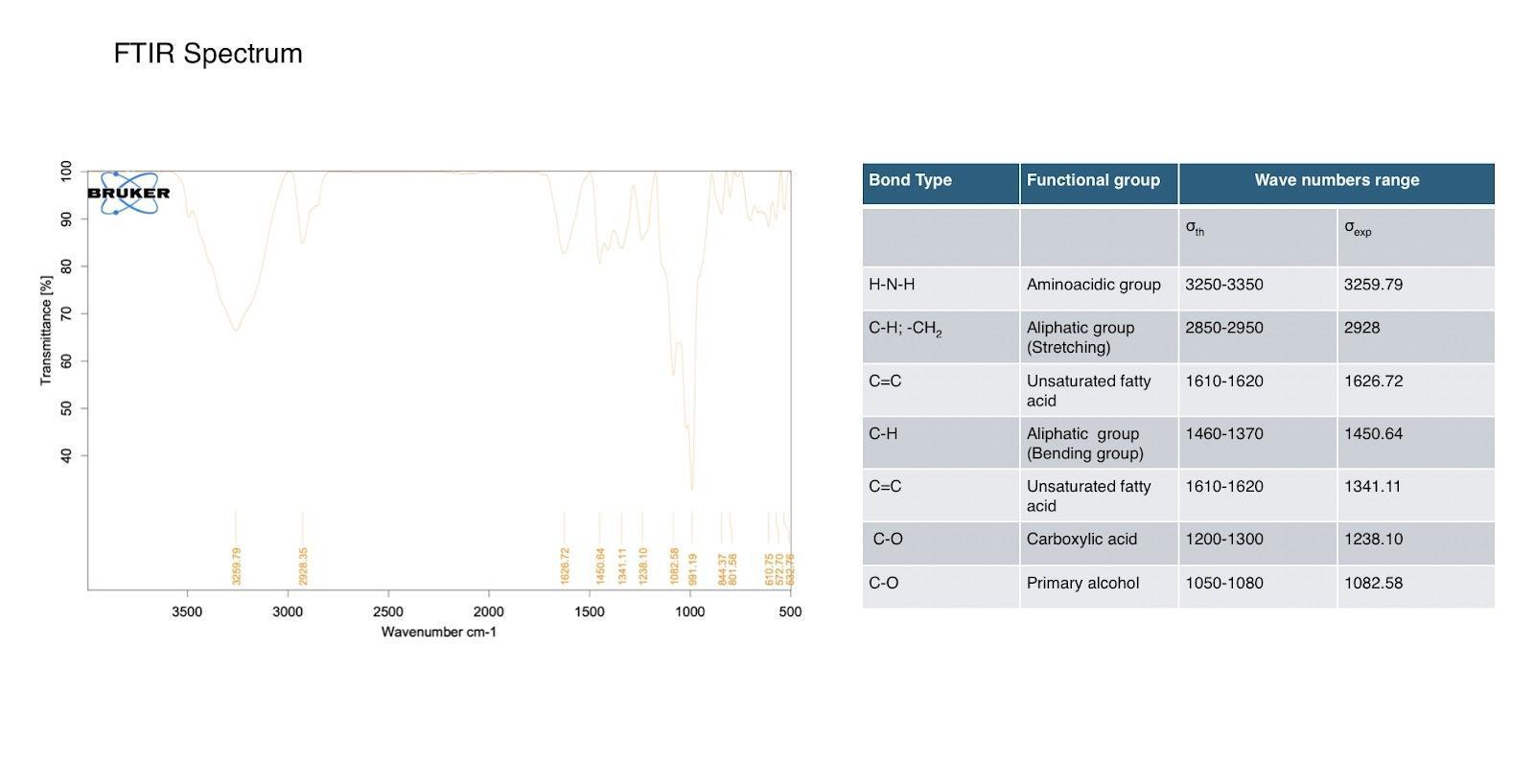
**Figure 1: Material**



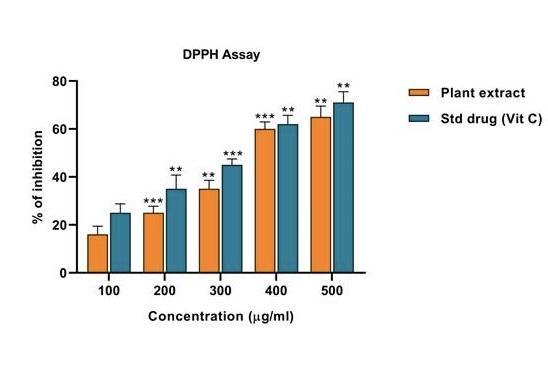
**Figure 2 Concentration vs % of inhibition**



**Figure 3: MTT Assay KB cells**



**Figure 4: FTIR spectrum**



**Figure 5: DPHH assay**

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