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Antioxidant Potential of *Cordyceps militaris* Mycelium: A Comparative Analysis of Methanol and Aqueous Extracts

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Reactive oxygen species (ROS) are the metabolic byproducts of various biochemical processes of the cell. However, if the production of ROS is significantly higher compared to the cell's defence mechanism, it results in oxidative stress. Accumulation of ROS and subsequent oxidative stress can lead to a range of harmful effects on the cell, including cellular damage, inflammation, and disruptions in normal cellular functions. These effects can ultimately contribute to the development of numerous health conditions. To counteract this, it is important to supplement the body with natural antioxidants. The medicinal mushroom Cordyceps militaris, has been used for centuries for its potential health benefits and has been shown to possess antioxidant capabilities. The presented study evaluates and compares the total antioxidant capacity of two mycelium extracts of Cordyceps militaris made from methanol and water based on multiple assays. The investigation further noted that the aqueous extract exhibited greater phenolic content with a concentration of 8.50 mg of GAE/g. In contrast, the methanol extract showed a higher flavonoid content of 7.13 mg of CAE/g. The study's findings revealed that methanol and aqueous extract possess antioxidant capability that can neutralize ROS and help reduce oxidative stress.

Keywords: Antioxidant; Cordyceps militaris; Oxidative stress; ROS; Reducing power.

Cellular metabolism involves a complex cascade of metabolic reactions. ROS were initially recognized as toxic compounds produced through various biological processes such as respiration, metabolism, and inflammation. However, recent developments have shown that ROS plays significant roles in cellular signaling, immune responses, and regulation of diverse cellular processes. Studies have demonstrated that oxidative stress may occur when ROS production and accumulation exceed normal threshold values or when their levels are not adequately controlled. This can result in cellular damage and contribute to developing health concerns and ageing processes. Members of the ROS family are characterized by the presence of unpaired electrons, which make them highly reactive and able to interact with various biomolecules (protein, DNA, lipid). Therefore, due to ROS, there is always a risk of damage to these components. The ROS family consists of significant members, such as superoxide anion ($O_2^{\bullet-}$) a byproduct of cellular respiration [1,2], Hydroxyl radical ($^{\bullet}OH$) formed through the Fenton or Haber-Weiss reactions [3], Peroxyl radical

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(ROO•) formed during the propagation step of lipid peroxidation [4], Hydrogen peroxide (H2O2) formed by the dismutation of superoxide anion [5,6], singlet oxygen ($^1O2$) and peroxynitrite ($ONOO^-$) formed through energy transfer from excited photosensitizers and reaction between superoxide anion and nitric oxide (NO) respectively [7].

Endogenous enzymes, including glutathione peroxidase, thioredoxin reductase, peroxiredoxins catalase, glutathione reductase, paraoxonase, methionine sulfoxide reductases and superoxide dismutase, are naturally synthesized by cells to counteract the harmful effects of free radicals. These enzymes work together to neutralize ROS and prevent their harmful effects on cellular components [8,9]. In addition to enzymatic antioxidants, cells also produce various non-enzymatic antioxidant molecules, including vitamins (E and C), to neutralize the harmful effects associated with ROS [10]. In recent years the health-promoting properties of natural antioxidants obtained from various sources, such as mushrooms, medicinal plants, fruits, vegetables, and others, have gained substantial attention due to their potential health benefits. These natural antioxidants, such as polyphenols, carotenoids, and flavonoids, have the ability to scavenge ROS and shield the body against oxidative damage and related diseases [11]. Humans have consumed mushrooms as a food source from ancient times [12]. They have been valued for their unique flavours, textures, and aroma [13]. In recent years, medicinal mushrooms have gained much importance and popularity among the public and the scientific community [14]. As per the "World Health Organization", more than 75 % of the global population now utilizes mushrooms, herbs, and other herbal medicines to treat various diseases. In the past two decades, various studies have elucidated mushrooms' nutritional and nutraceutical characteristics, demonstrating them to be promising reservoirs of bioactive metabolites [15]. They are reported to contain various bioactive molecules like Beta-glucans, Ergosterol, Lentinan, Triterpenoids, Amino acids, Lectins, Cordycepins, Enzymes and Polysaccharides [16].

In addition, mushrooms are recognized as a plentiful source of Ergothioneine, Glutathione, Selenium, Vitamins, Phenolic compounds, and Carotenoids considered strong natural antioxidants [17]. *Cordyceps militaris,* an edible medicinal mushroom, has a rich traditional history of use in different regions worldwide particularly in eastern Asia for its potential health benefits and medicinal values [18]. *Cordyceps militaris* differs from the other species in that it grows on insect larvae and various other substrates, including rice and grains, making it easier to cultivate in laboratories [19]. In traditional medicine, it has been used for its potential benefits for the respiratory, immune system, energy and endurance, anti-inflammatory, and sexual health, as well as for its antioxidant properties [20]. The traditional method of cultivating *Cordyceps* fruiting bodies can take several months, making it impractical for large-scale production [21]. However, submerged fermentation of *Cordyceps* mycelial biomass is a more efficient alternative, as it requires less time and space with minimum risk of contamination [22]. This investigation assessed the antioxidant activity of the most widely used solvent systems for extract preparations, i.e., methanol and water. The study incorporated multiple assays to evaluate the total antioxidative status of these two extracts.

MATERIAL AND METHODS

Chemicals and Microbial strain

A strain of *Cordyceps militaris* has been acquired from LJ University, Ahmedabad, India, and all the analytical-grade chemicals employed in the study were procured from Himedia located in Mumbai, India.

Culture Conditions

An Erlenmeyer flask (250 mL), having 100 mL of basal media (1.5% glucose, 0.1% w/v Dipotassium phosphate, 0.5% peptone, 0.3% potassium dihydrogen phosphate, 0.05% magnesium sulfate and 0.05% sodium chloride) wear used to culture *Cordyceps militaris*. The flasks were kept for 10 days in static conditions at 20°C. Afterwards, the mycelia-containing culture media were filtered with the help of the Whatman #4 filter paper. The harvested mycelia were subjected to three washes with autoclaved deionized water, and until a consistent dry weight was obtained, it was oven-dried at 45°C.

Extract Preparation

To obtain the extract, 5 grams of mycelium powder are added to the solvent (100 mL of either water or methanol), and the reaction

mixture is placed on an orbital rotary shaker that is set to rotate at a speed of 120 rpm at room temperature (RT) under light protected area. The solution was subjected to centrifugation (6500 rpm + 10 minutes). The obtained residue was further used for two subsequent extractions following the same procedure. A rotary evaporator was used to concentrate the extract. The final concentration of the extracts, 50 mg/mL, was achieved by dissolving dried extracts in their respective solvents.

DPPH assay for scavenging activity

The previously reported approach is employed to evaluate the scavenging ability of the *Cordyceps militaris* mycelium in methanol and aqueous extracts, with a few minor adjustments [23]. To dissolve DPPH and make a 0.1 mM final solution, methanol was utilized as the solvent. To reaction mixture was prepared by mixing mycelium extracts and DPPH solution in a 4:1 ratio (4 mL extract + 1mL DPPH). After vigorously shaking, the solution was left undisturbed for 30 minutes at RT. This is followed by the measurement of optical density at 517 nm in a spectrophotometer (LABMAN LCD LMSP-UV1000B)

ABTS assay for scavenging activity

The ABTS● scavenging capability of the mycelium extract is carried out using previously reported methods with some alterations [24]. An equal volume of, ABTS solution (7 mmol/L) and potassium persulfate solution (2.45 mmol/L) were mixed to make ABTS●+ reagent (kept in the dark conditions for 12-16 hours at room temperature before use). To regulate the optical density of the ABTS●+ reagent to the required level, a 7.4 pH phosphate-buffered saline (5 mmol/L) was step by step added until the optical density reached a value of 0.70±0.02 at 730 nm.

Hydroxyl (•OH) radical assay for scavenging activity

A modified version of an earlier reported method was used to analyze the capacity of the mycelium extract to scavenge •OH radicals [25,26]. 1 mL of the methanolic mycelium extract mixed with 1.5 mM ferrous sulphate (1 mL), 20 mM sodium salicylate (0.3 mL), and 6 mM hydrogen peroxide (0.7 mL). The resultant solution was thoroughly mixed, and at 562 nm, its optical density was measured.

Reducing assay

We utilized an alternative approach based on prior research [27,28]. To initiate the experiment, we combined 2.5 mL of mycelium extract with an equivalent volume of 0.2 M phosphate buffer (pH 6.6). Next, we introduced 2.5 mL of a 1% potassium ferricyanide solution in phosphate buffer to the reaction mixture. Following incubation at 50°C for 20 minutes, this solution underwent treatment with 2.5 mL of 10% trichloroacetic acid and centrifuged at a speed of 5500 rpm for 10 minutes. Subsequently, we added 0.5 mL of a 1% ferric chloride solution to the collected supernatant, followed by the introduction of 2.5 mL of distilled water. At 700 nm, the optical density of the resulting solution was measured.

CUPRAC assay

The method used to evaluate the ability of mycelium extract to reduce cupric ions (Cu2+) was based on a previous study [29]. To conduct the assay, add methanolic mycelium extract (0.25 mL) in a tube and mix with equal volumes (0.25 mL) of three reagents, namely copper (II) chloride, ammonium acetate (1M), and neocuproine (7.5 mM). Obtain the desired final reaction mixture (2 mL) and add distilled to make up the volume. After incubation (30 minutes at RT), at 450 nm optical density was measured.

Metal chelation assay

The chelation property of the mycelium extract was studied with a previous approach with small modifications [30]. For the assay, 2.0 mL of methanolic mycelium extracts were mixed with 0.05 mL of 2 mM ferrous chloride in a tube. The reaction was initiated by the addition of 0.5 mM ferrozine (2 mL) to the mixture. The resulting solution was thoroughly mixed for 8 minutes, and at 562 nm its optical density was recorded.

Total phenolic content

The mycelium extracts were assessed for their total phenolic content using the previously reported method [31]. To determine the phenolic content, Add Folin-Ciocalteu's phenol reagent (1 mL) in a tube containing mycelium extract (1 mL). It is followed by the addition of 35% saturated sodium carbonate and the final volume is adjusted using deionized water to 10 mL. The reaction mixture was carefully placed in a light-

protected environment and allowed to incubate for 90 minutes. The optical density of the resultant solution was recorded at 725 nm. The results of the experiment were reported and quantified in terms of gallic acid equivalents (GAE).

Total flavonoid content

The mycelium extracts are investigated for their flavonoid content using a modified version of a previously reported method [32]. In the assay, a reaction mixture was prepared by combining mycelium extract (0.25 mL) and 5% sodium nitrite (75 µL). After incubation of 4 minutes add 10% aluminum chloride (150 µL) and mix properly. The resulting solution was again incubated for 4 minutes. Afterwards, 1 M sodium hydroxide (0.5 mL) and at 510 nm its optical density was measured. The results of the experiment were reported and quantified in terms of catechin equivalents (CAE).

RESULTS AND DISCUSSION

Scavenging assay

Natural compounds are always a rich source of antioxidant molecules and DPPH assay is the most widely used protocol for such analysis.

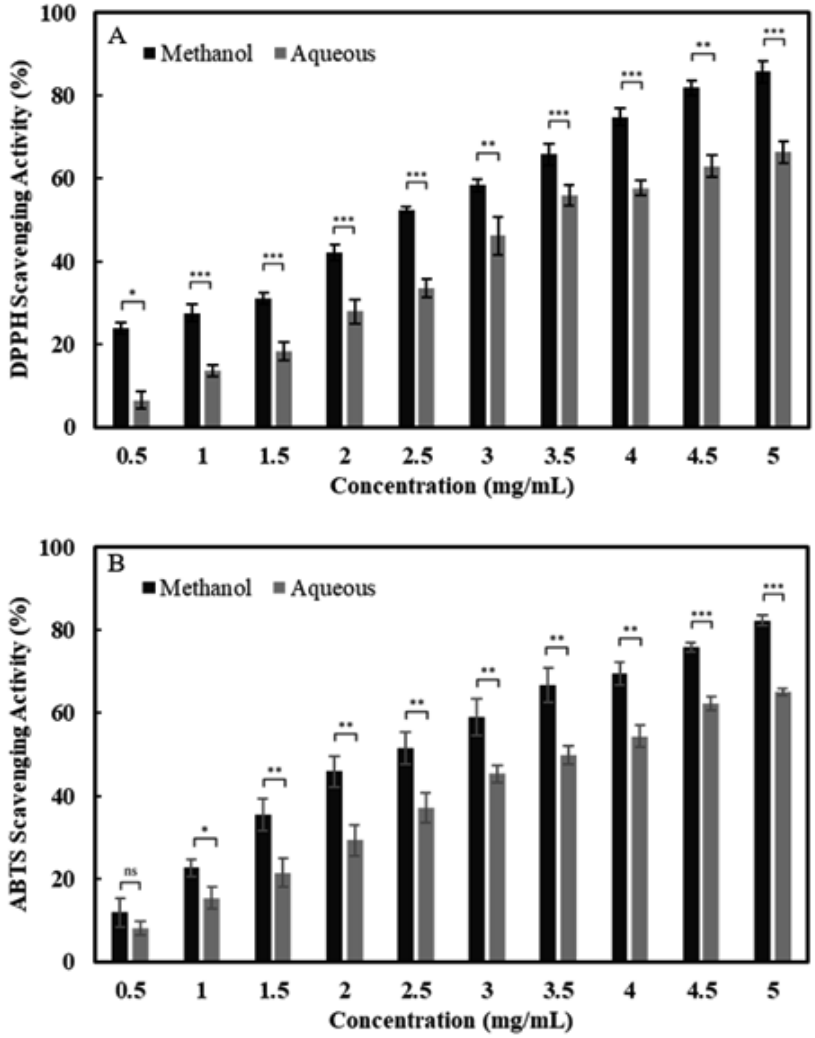

Fig. 1. (A). DPPH scavenging activity. (B) ABTS scavenging activity. The results are presented as mean ± standard deviation (n=3), Significant difference calculated with ANOVA (^{ns}non-significant, *p = 0.05, **p = 0.01, ***p = 0.001)

The assay used the ability of a molecule to provide hydrogen or an electron to a radical which can be estimated with the help of a reduction in the absorbance at 517 nm. Figure 1.1 (A) depicts the scavenging effects of the mycelium extract of *Cordyceps militaris* concerning the DPPH radical. The results demonstrate increased scavenging activity for both the mycelium extracts with increasing concentrations. The methanol extract exhibited the highest percentage of inhibition, achieving 85.37%, while the highest level of inhibition was observed with the aqueous extract, 66.41%. However, compared to Vc at a 0.1 mg/mL concentration, the aqueous and methanolic extracts displayed lower maximum percentage inhibition values.

The study observed that the scavenging activity of the methanol and aqueous extracts against DPPH radicals was evaluated, yielding distinct results. The methanolic extract exhibited

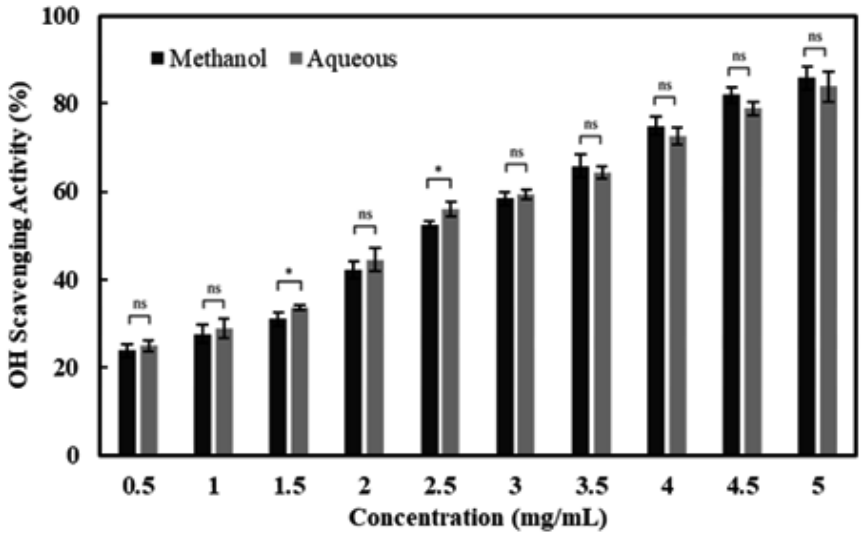

Fig. 2. OH. Radical scavenging activity, the results are presented as mean ± standard deviation (n=3), Significant difference calculated with ANOVA ([ns]non-significant, *p = 0.05, **p = 0.01, ***p = 0.001).

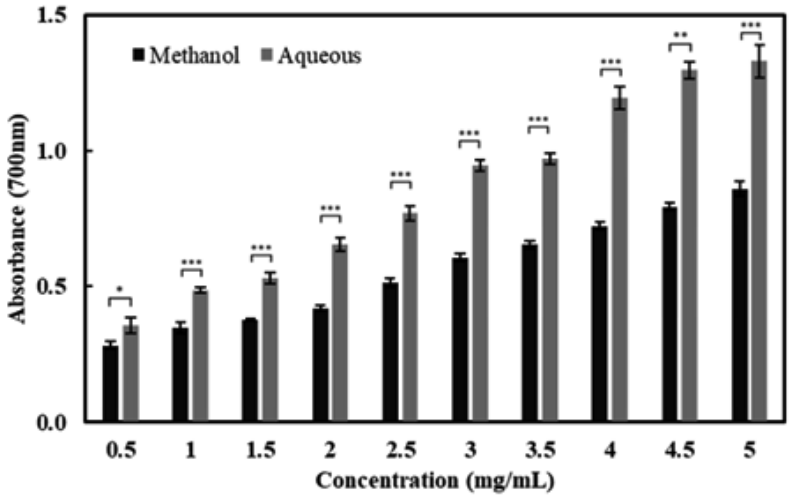

Fig. 3. Results of reducing power assay, the results are presented as mean ± standard deviation (n=3), Significant difference calculated with ANOVA ([ns]non-significant, *p = 0.05, **p = 0.01, ***p = 0.001).

the highest scavenging activity, as evidenced by its EC50 value of 2.92 mg/mL. On the other hand, the aqueous extract demonstrated a weaker scavenging activity, with a higher EC50 value of 3.76 mg/mL. A separate investigation also explored the ABTSÏ%+ scavenging activity of both extracts, demonstrating a concentration-dependent scavenging activity. The methanol extract displayed the highest percentage of scavenging activity, with an EC50 value of 2.43 mg/mL, while the aqueous extract showed an EC50 value of 3.68 mg/mL. Statistical analysis indicated a significant variation ($p<0.05$) in the activities of the methanol and aqueous extracts, as illustrated in Figure 1.1 (B).

Antioxidants are necessary for the human body to counteract and neutralize the harmful effects of free radicals, which are produced in endogenous biochemical reactions as well as exogenous sources like pollution, radiation, and cigarette smoke. When these free radicals interact with cells, they can cause oxidative stress by damaging various biomolecules like DNA damage: ROS can directly interact

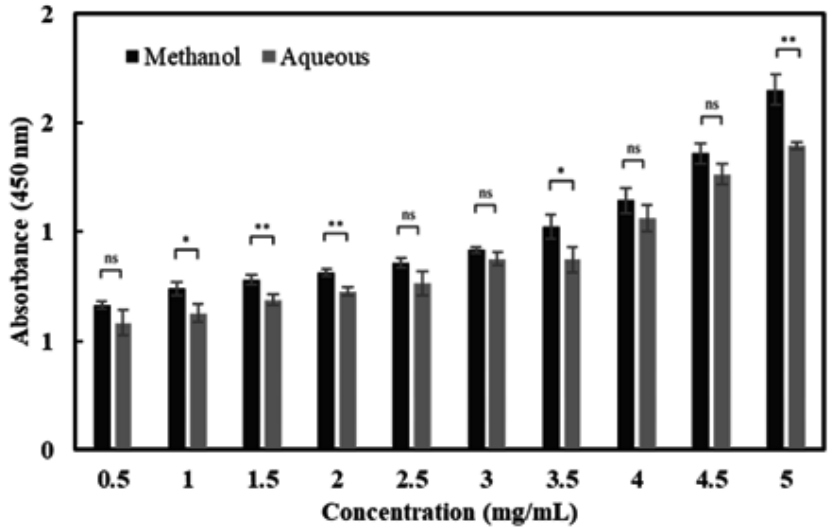

Fig. 4. Result of CUPRAC assay, the results are presented as mean ± standard deviation (n=3), Significant difference calculated with ANOVA (nsnon-significant, *p = 0.05, **p = 0.01, ***p = 0.001).

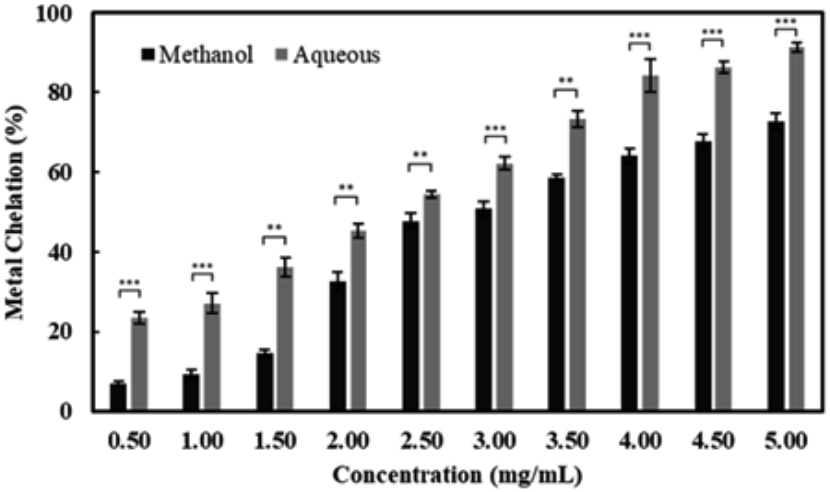

Fig. 5. Ferrous ion chelating activity, the results are presented as mean ± standard deviation (n=3), Significant difference calculated with ANOVA (nsnon-significant, *p = 0.05, **p = 0.01, ***p = 0.001).

with DNA molecules, leading to the formation of DNA adducts and strand breaks [33]. Protein damage, ROS can oxidize amino acid residues within proteins, leading to protein carbonylation, formation of protein adducts, and cross-linking. These oxidative modifications can disrupt protein structure and function, impair enzymatic activity, and affect protein-protein interactions [34]. Lipids damage, ROS can initiate lipid peroxidation, a chain reaction that results in the oxidation of polyunsaturated fatty acids within cell membranes [35]. The cumulative damage to DNA, proteins, and lipids caused by oxidative stress can disrupt cellular homeostasis and contribute to the progression of various diseases, including cardiovascular diseases, neurodegenerative disorders, cancer,

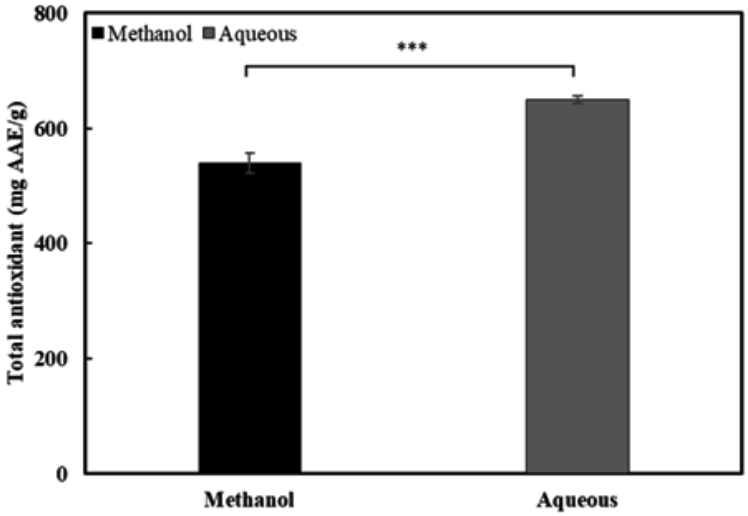

Fig. 6. Results of phosphomolybdenum assays, the results are presented as mean ± standard deviation (n=3), Significant difference calculated with ANOVA ([ns]non-significant, *p = 0.05, **p = 0.01, ***p = 0.001).

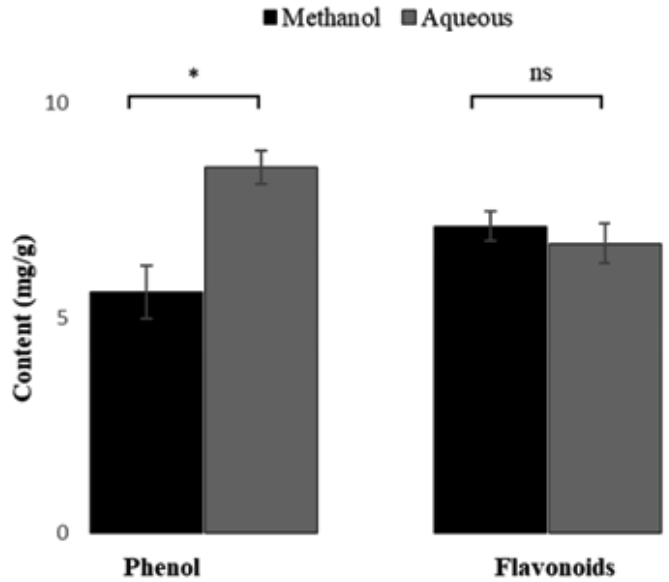

Fig. 7. Total phenolic and flavonoid contents, the results are presented as mean ± standard deviation (n=3), Significant difference calculated with ANOVA ([ns]non-significant, *p = 0.05, **p = 0.01, ***p = 0.001).

and age-related conditions. Antioxidants work by neutralizing free radicals and reducing their harmful effects by donating electrons that stabilize the free radicals, thus preventing further cell damage. Such electron donors as phenolic compounds, cordycepin, and ergosterol are responsible for the scavenging activity of the mycelium extracts of *Cordyceps militaris*. Numerous studies have reported on the antioxidant activity of various medicinal mushrooms, *Ganoderma lucidum* (Reishi) [15,36,37]. *Lentinula edodes* (Shiitake) [38–40], *Pleurotus eryngii* [41–43] and *Hericium erinaceus* (Lion's Mane) [44,45]. These mushrooms have been studied for their potential health benefits and their antioxidant activity against DPPH radicals. The variations in the % ABTS scavenging values are due to the differences in the techniques employed to extract the bioactive compounds, the types of mushrooms studied, and the concentration of extracts used. However, one of the previously reported studies highlighted the difference in the scavenging activity due to drying methods [46]. They studied the effect of sun-drying, freeze-drying, hot air, and microwave-drying. The study achieved the highest scavenging activity in the extract dried by microwave at 700 W.

OH• radical scavenging activities

OH• is widely acknowledged as an extremely harmful oxygen species that has the ability to cause adverse effects on essential biomolecules such as proteins, deoxyribonucleic acid, carbohydrates, and lipids. Eliminating OH• radicals plays a vital role in averting damage induced by oxidative stress to cells. The capability of mycelium extracts to scavenge hydroxyl radicals was evaluated by comparing it with salicylic acid. The concentration-dependent increase in scavenging efficacy was observed for both methanol and aqueous mycelium extracts of *Cordyceps militaris*, with values rising from 23.80% to 85.80% and from 24.90% to 83.88%, respectively, over the concentration range of 0.5-5 mg/mL. Based on the EC50 values, the aqueous extract demonstrated a slightly higher OH• radical scavenging activity compared to the methanol. The EC50 value for the aqueous extract was 2.23 mg/mL, while for the methanol extract, it was slightly higher at 2.38 mg/mL as depicted in Figure 1.2. Various factors, such as the extraction method, the solvent used, the extract's concentration, and the mushroom species, can influence the hydroxyl scavenging activity of the mycelium extract [47]. Different extraction methods, such as aqueous or organic solvent-based extraction, may yield different results regarding hydroxyl scavenging activity [48]

Ferric cyanide (Fe3+) reducing antioxidant power assay

Antioxidants can donate electrons to ferric (Fe3+) ions in the assay, measuring reducing power and converting them to ferrous (Fe2+) ions. This conversion can be quantified using spectrophotometry at 700 nm. Antioxidants have a vital function in halting free radical chain reactions by actively donating electrons to species that cause oxidative stress. The reducing capacity of methanol and aqueous mycelium extracts from *Cordyceps militaris* demonstrated a concentration-dependent pattern within the range of 0.5-5 mg/mL. Figure 1.3 illustrates the reducing power of both extracts. However, these values were relatively lower compared to the value of Vc, which was 1.639 at a concentration of 1.0 mg/mL.

Cupric ion antioxidant capacity assay

This assay is a widely employed technique for assessing the antioxidant capability of extracts to reduce copper (II) ions to copper (I) ions in the presence of neocuproine. The results of the current study revealed a concentration-dependent reduction in Cu^{2+} ions by mycelium extracts of *Cordyceps*, suggesting potential antioxidant activity. The findings showed that both methanol and aqueous extracts of *Cordyceps militaris* mycelium exhibited cupric ion-reducing abilities, with absorbance values ranging from 0.66 to 1.65 for methanol extracts and 0.58 to 1.39 for aqueous extracts at concentrations between 0.5 to 5 mg/mL. These results indicate that higher concentrations of the extracts resulted in higher cupric ion-reducing power, suggesting a concentration-dependent effect. However, it is noteworthy that the observed values were lower than the control value of 1.682 observed at 0.2 mg/mL, indicating that the reducing power of the extracts at higher concentrations (0.5 to 5 mg/mL) was not as strong as the reference as depicted in Figure 1.4. The findings of this study are in line with prior research in the field that has reported the antioxidant activity of *Cordyceps* mycelium extracts. The observed concentration-dependent reduction in Cu^{2+} ions by the extracts

suggests the presence of bioactive components with potential antioxidant properties. However, it is important to note that this assay measures the reducing power of antioxidants against copper ions only, and other mechanisms of antioxidant activity may also be at play in *Cordyceps* mycelium extracts. The observed decrease in cupric ion-reducing abilities of the extracts at higher concentrations compared to the control at 0.2 mg/mL raises some questions. At higher concentrations, the extracts may have exhibited pro-oxidant activity, leading to a decrease in their cupric ion-reducing abilities. This phenomenon, known as the pro-oxidant effect, has been reported in some studies where high concentrations of certain antioxidants can act as pro-oxidants and induce oxidative damage under certain conditions. Further investigation is needed to determine if this is the case with *Cordyceps* mycelium extracts and to elucidate the underlying mechanisms.

Ferrous ion chelating ability

Studies demonstrate the chelating effect of the extracts on ferrous ions (Fe^{2+}) is indeed a critical factor in mitigating or reducing oxidative damage caused by ROS within the body. The results showed that the chelating activity of both extracts was concentration-dependent. The findings revealed that both extracts of *Cordyceps militaris* mycelium exhibited ferrous ion chelating activity, with inhibition percentages ranging from 2.16% to 75.92% for methanol extracts and 46.75% to 94.52% for aqueous extracts. Furthermore, it is of utmost importance to recognize that the inhibition percentage of the reference chelating agent, EDTA, was higher (84.67% at 0.05 mg/mL) than the extracts, indicating that EDTA had a stronger chelating agent against ferrous ions. Notably, the aqueous extracts of *Cordyceps militaris* showed a significantly higher chelating ability against ferrous ions compared to the methanol extracts, as evidenced by the lower EC50 value of 3.49 mg/mL for aqueous extracts compared to 5.37 mg/mL for methanol extracts depicted in Figure 1.5. This suggests that the aqueous extracts of *Cordyceps militaris* may be more effective in sequestering ferrous ions and preventing ROS generation via the Fenton reaction than the methanol extracts. The findings of this study align with prior research that has reported the metal-chelating activity of *Cordyceps militaris*. The observed concentration-dependent chelating activity of *Cordyceps militaris* extracts against ferrous ions suggests that the bioactive components responsible for this activity may be present in varying concentrations in the extracts, and further investigation is needed to identify and characterize these components. The findings of this study have potential implications for the use of *Cordyceps militaris* extracts as a natural source of metal chelators for the prevention or mitigation of oxidative damage in biological systems. The occurrence of oxidative stress is associated with a range of pathological conditions, including cancer, neurodegenerative and cardiovascular diseases. Consequently, the potential of *Cordyceps militaris* extracts to chelate ferrous ions and counteract the adverse effects of ROS.

Phosphomolybdenum Assays: Measuring Total Antioxidant Activity

The total antioxidant capacity of an herbal extract is evaluated using phosphomolybdenum Assays. It is a widely used assay that relies on the potency of the extract's antioxidant components to reduce Mo(VI) to Mo(V). The reduction process leads to the formation of a green-coloured complex, which can be measured and quantified using spectrophotometry at a wavelength of 695 nm. Based on the findings of the study, the aqueous extract exhibited a higher total antioxidant activity. The aqueous extract demonstrated a value of 650.53 mg AAE/g, indicating its superior antioxidant potential. In comparison, the methanol extract had a value of 540.38 mg AAE/g as depicted in Figure 1.6. The findings suggest that the aqueous mycelium extract has a higher capacity to reduce Mo(VI) to Mo(V), which indicates a stronger antioxidant potential. The higher total antioxidant activity observed in the aqueous extract may be due to water-soluble antioxidant compounds that can effectively reduce Mo(VI) to Mo(V). These water-soluble compounds may include phenolic compounds, flavonoids, and other polar antioxidants readily extractable in water. It is also possible that the extraction process with methanol may not have efficiently extracted all the water-soluble antioxidants, resulting in lower total antioxidant activity in the methanolic extract.

However, the methanol extract may contain other lipophilic antioxidants that are not as readily extractable in water. Methanol is a

polar organic solvent that is capable of extracting lipophilic compounds, such as carotenoids [49], tocopherols [47], and other non-polar antioxidants [50], which may be accountable for the activity of the mycelium methanolic extract. However, the phosphomolybdenum assay, being water-based, may not be as effective in capturing the lipophilic antioxidants present in the methanol extract, leading to a lower total antioxidant activity value.

Determination of phenolic and flavonoid compounds

The existence of phenolics and flavonoid compounds within the herbal extracts mostly contributes to the observed antioxidant activities. These macromolecules can neutralize ROS and free radicals by donating electrons, thereby mitigating oxidative stress, and protecting cells from damage. Failure to manage free radicals and ROS can lead to various health issues. Figure 1.7 illustrates the presence of these macromolecules in the methanol and aqueous mycelium extract of *Cordyceps militaris*. According to the study, it was observed that the aqueous extract exhibited a higher phenolic content, measuring 8.50 mg of GAE/g, in comparison to the lower phenolic content of the methanol extract, which measured 5.60 mg of GAE/g. In contrast, the study revealed that the methanol extract displayed a higher flavonoid content, measuring 7.13 mg of CAE/g, in comparison to the aqueous extract, which measured 6.73 mg of CAE/g. The statistical analysis of the phenolic content of both extracts demonstrated a significant variation ($p < 0.05$). However, no statistical significance was observed in the flavonoid content.

There are very few reports on the *Cordyceps* species. However, one such study reported the difference in the phenolic and flavonoid content in *Cordyceps sinensis* (CS) in different solvent systems (aqueous, 50%, 75%, and 100% alcohol). "According to the study conducted, the CS50% Alcohol extract exhibited the highest phenolic content (15.1 ± 0.67 mg/g), while the CS100% Alcohol extract contained the highest amount of flavonoids (19.3 ± 0.3 mg/g). In the case of the aqueous extract, the phenolic content was determined to be 13.8 ± 0.28 mg/g, and the flavonoid content was found to be 11.3 ± 0.33 mg/g [51]". Another study also determined the total phenols and flavonoid content in the fruiting body of *Cordyceps militaris* using methanol extract [52]. The study observed that the total phenols and flavonoids present in the extract are 39.52 ±0.040 mg/g and 1.56 ±0.039 mg/g, respectively.

In this particular investigation, the discrepancy in the quantity of phenolic and flavonoid compounds observed in the two extracts may be due to the utilization of distinct solvents during the extraction process. Methanol and water have different polarities and solubility properties that can affect the efficacy of extraction from mycelium. Phenolic compounds are more soluble in water, which results in their more efficient extraction in aqueous extracts. Conversely, the higher content of flavonoids in methanol is due to their better solubility in methanol. It is crucial to acknowledge that the antioxidant property of the mycelium extracts of *Cordyceps militaris* cannot be exclusively credited to phenolic and flavonoid compounds, as other bioactive contents in the mycelium could support the total antioxidant activity. Nonetheless, the findings also revealed that both methanol and aqueous extracts contain a substantial amount of phenolic and flavonoid compounds. These compounds are recognized for their antioxidant potential and have the ability to contribute towards the health benefits associated with this herbal extract.

CONCLUSION

The study investigated the total antioxidant potential in the mycelium extract (methanol and water) of *Cordyceps militaris*. The finding of the study demonstrated that both extracts showed antioxidant activity. The activity is found to be concentration dependent as concentration increases it rise in activity was observed. The study also concluded that the activity is greatly influenced by the nature of the assay and the choice of solvent. There is a direct correlation between the bioactive molecules that are responsible for the activity and the solvent used for extraction. In the presented study, higher phenolic content was observed in the mycelium aqueous extract while on the other hand, higher flavonoid content was present in the methanolic extract. Both compounds have gained recognition for their remarkable antioxidant properties. These results support the traditional use of *Cordyceps militaris* as a

medicinal mushroom with potential health benefits. The research contributes to the growing body of knowledge regarding natural antioxidants derived from mushrooms and their potential applications in preventing oxidative damage and related diseases. Further studies can explore the specific bioactive compounds that are actively involved in the antioxidant activity observed in *Cordyceps militaris* mycelium extracts and investigate their mechanisms of action.

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Conflict of Interest

There is no conflict of interest.

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