



STUDIES ON ANTIBACTERIAL ACTIVITY OF *CORDYCEPS MILITARIS* (L.) LINK

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ABSTRACT

Antibacterial activity of aqueous and methanolic extracts of *Cordyceps militaris* was determined *in-vitro* against two pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*) following agar well diffusion method using different concentrations (25, 50, 75 and 100%). Aqueous and methanolic extracts showed potent antibacterial activity against selected strains. Methanolic extract showed a maximum inhibitory effect against growth of each of the test bacterium. There is a need for further studies to isolate and characterize the antibacterial moieties in this fungus for practical disease control measures.

KEYWORDS: *Cordyceps militaris*, antibacterial, *in-vitro*, *Staphylococcus aureus*, *Escherichia coli*.



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INTRODUCTION

A medicinal herb of long and illustrious history, *Cordyceps* is a fungus of subphylum Ascomycotina, class Pyrenomycetes, order Clavicipitales and family Clavicipitaceae¹. *Cordyceps*, a macrofungus parasitic on insects, is used as a source of functional food and medicines. Some members of the genus *Cordyceps*, for example *C. sinensis*, *C. ophioglossoides*, *C. cicadae* and *C. militaris* are valuable materials in traditional Chinese medicine². *Cordyceps militaris*, a medicinal fungus that contains many kinds of active compounds is now used for a variety of medicinal purposes³⁻⁴. It has benefits for the human body, including beneficial effects on the circulatory, immune, respiratory and glandular system. It is commonly used in the treatment of hyperglycemia, hyperlipidemia⁵⁻⁶, renal dysfunction and liver diseases⁷. Recently, several studies demonstrated that extracts of *Cordyceps militaris* have multiple pharmacological actions which included anti-inflammatory activity⁸, improvement of insulin resistance, insulin secretion and tumor suppression activity⁶. In the present study, antibacterial activity of aqueous and methanolic extracts of *C. militaris* collected from Distt. Shimla and adjoining areas have been investigated.

MATERIALS AND METHODS

Materials used

Materials used in the present study were fruiting bodies and pure cultures of *C. militaris* and two bacterial pathogens (*Escherichia coli* and *Staphylococcus aureus*). Pathogenic strains of bacteria were procured from IGMC, Department of Microbiology, Shimla.

METHODOLOGY

Survey and collection

The fruiting bodies of *C. militaris* were collected from Glen forest (Shimla) H.P. and its vicinity during June-September 2011-2013. Collection sites were visited regularly after every spell of rain. The fruiting bodies were collected carefully with the help of forceps.

Isolation of pure culture of *Cordyceps militaris*

The cultures were raised from the stipe and stroma portion of healthy, sun-dried and fresh specimens. The specimens were first washed with distilled water and then the tissue from the stipe and stroma portion were cut with the help of a sterilized blade. The bits of tissue (2-3 mm) were taken up with a sterilized forceps and dipped in 0.1% mercuric chloride solution for 5-10 seconds. Now the tissue was placed on filter paper to remove the excess moisture. The small bits of *Cordyceps* tissues were then transferred aseptically into the petriplates containing potato-dextrose agar (PDA) medium with the help of a sterilized forceps. These were then incubated at 25°C for at least 8-10 days and observed regularly for appearance of culture. The actively growing mycelial colonies were sub cultured to obtain pure cultures.

Preparation of crude mushroom extract

Aqueous and methanolic extracts of experimental fruiting bodies were prepared following Indian Pharmacopoeia[9]. The fresh fruit bodies were dried in shade conditions and the dried material (50 g) was pulverized in a blender to get a coarse powder and soaked separately in 300 ml of distilled water and methanol in Erlenmeyer flask. The flasks were covered with aluminum foil and allowed to stand for 7 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator¹⁰⁻¹¹. The extracts were collected and stock solution of conc. 10 mg/ml was prepared.

Screening of extract of *Cordyceps militaris* for antibacterial activity

Screening of mushroom extracts (aqueous and methanolic) of *Cordyceps militaris* was done using agar well diffusion method. Nutrient agar medium (Beef extract 1 g, yeast extract 2g, sodium chloride 1g, peptone 5g, agar 20g, distilled water 1000 ml) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into petriplates. Bacteria were grown in nutrient broth for 24 hours. A 100 µl of

bacterial suspension was spread on each nutrient agar plates. One agar well of 8 mm diameter was prepared with the help of sterilized stainless steel cork borer in each petriplate. The well in each plate was loaded with 25%, 50%, 75% and 100% concentration of prepared extracts of *Cordyceps militaris*. The plates were incubated at $37 \pm 2^\circ\text{C}$ for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring

the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all three replicates and the average values were tabulated. Percentage inhibition of growth of bacterial microorganisms was calculated after subtracting control from the values of inhibition diameter using control as standard¹²

$$\text{Percentage of growth inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Control = average diameter of bacterial colony in control.

Test = average diameter of bacterial colony in treatment sets¹³.

RESULTS AND DISCUSSION

Macroscopic characters

The fruiting bodies of *Cordyceps militaris* (L.) Link were creamish white in colour. Fruiting body shows association with insect larvae and pupae. The associated insect's body becomes mummified by the growth of the mycelium. The mycelium of the fungus forms fruiting bodies, which interestingly, always emerges from the head of the insect. The size of fruiting bodies varies from 4.2 to 7.8 cm (Plate -1, a-b).

Pure culture isolation

Mycelial characteristics

Mycelial growth of *Cordyceps militaris* (L.) Link was longitudinally radial, aerial initially, creamish white, becoming densely matted and wooly in texture. As soon as the colony matures the colour of mycelium changes from creamish white to light brown. As the medium is completely consumed, the mycelia became increasingly mud-like and granular in texture. At approximately 16 days of growth or a bit later, the mycelium of *Cordyceps militaris* begins to form small nodules (perhaps sclerotia) at the centre on the surface of medium, appearing light brown while peripheral mycelia remain creamish white (Plate-1, c-d).

Antibacterial activity of *C. militaris* against *S. aureus* and *E. coli*

The aqueous and methanolic extracts of *C. militaris* were screened against *S. aureus* and *E. coli*. Stock solution was prepared by

making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution. Aqueous and methanolic extract of *Cordyceps militaris* showed considerable growth inhibition against two test bacteria in different concentrations (25% to 100%). Aqueous extract of *Cordyceps militaris* showed maximum inhibition of 17.64% and 22.74% at 100% concentration against *S. aureus* and *E. coli* respectively (Table 1 Plate 2 a-j). Methanolic extract exhibited maximum inhibition of 19.60% against *S. aureus* and 25.09% against *E. coli* at 100% concentration (Table 2 and Plate 2 k-t). It is evident from the results that aqueous and methanolic extracts of *C. militaris* showed maximum percent inhibition against *E. coli* and methanolic extract were more effective than aqueous extract against both the test bacteria (Fig.1A&B). The results of the present study are in agreement with the work of the earlier workers¹⁴⁻¹⁵ who have also reported strong antibacterial activity of methanolic extract of *G. lucidum* against gram negative (*E. coli*) and comparatively less activity against gram-positive (*S. aureus*) bacteria. Similar trend in antibacterial activity of methanolic extract of *Lactarius deliciosus*¹⁶, *Sparassis crispa*¹⁷, *Morchella esculenta*¹⁸ and *Ganoderma lucidum*¹⁹ have been reported against *S. aureus* and *E. coli*. Ramesh and Patter²⁰ have reported that extract of *Clavaria vermicularis* and *Marasmius oreades* offered more inhibition to gram-negative bacteria (*E. coli*

and *Pseudomonas aeruginosa*) as compared to gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). Neelam and Singh²¹ also reported the antibacterial potential of ethanolic extract of *Pleurotus florida* and *Pleurotus ostreatus*. Filipa *et al.*²² has reported that methanolic extract of *Cordyceps militaris* possesses antioxidant, antibacterial,

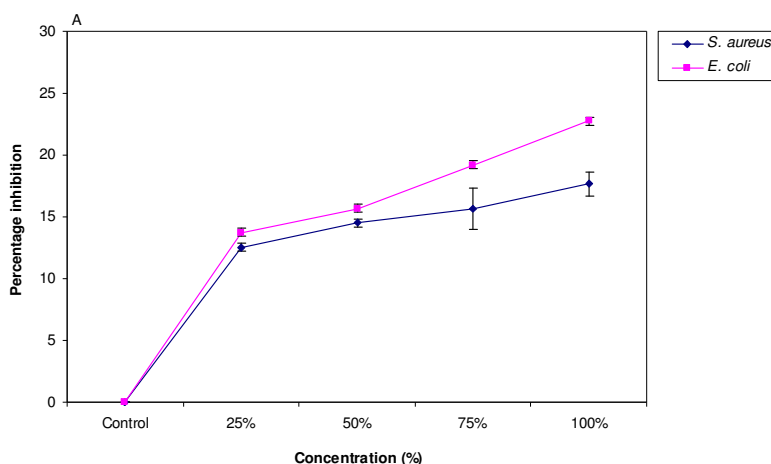
antifungal and antiproliferative properties. Many pharmaceutical substances with potent and unique health-enhancing properties have been isolated from medicinal mushrooms and distributed worldwide²³. Mushroom based products either from the mycelia or fruit bodies are consumed in the form of capsules, tablets or extracts²⁴.

Table 1
Percentage inhibition of growth of *S. aureus* and *E. coli* at different concentrations of aqueous extract of *C. militaris*.

Concentration of aqueous extract of <i>C. militaris</i> (in %)	Percentage inhibition of growth of test bacteria	
	<i>S. aureus</i>	<i>E. coli</i>
Control	0.00±0.00	0.00±0.00
25%	12.54±0.32	13.71±0.32
50%	14.50±0.32	15.68±0.32
75%	15.68±1.66	19.21±0.32
100%	17.64±1.00	22.74±0.32

Table 2
Percentage inhibition of growth of *S. aureus* and *E. coli* at different concentrations of methanolic extract of *C. militaris*.

Concentration of methanolic extract of <i>C. militaris</i> (in %)	Percentage inhibition of growth of test bacteria	
	<i>S. aureus</i>	<i>E. coli</i>
Control	0.00±0.00	0.00±0.0
25%	13.71±0.32	16.07±0.320
50%	15.68±0.32	18.03±0.32
75%	17.24±0.32	21.56±0.32
100%	19.60±0.66	25.09±0.32



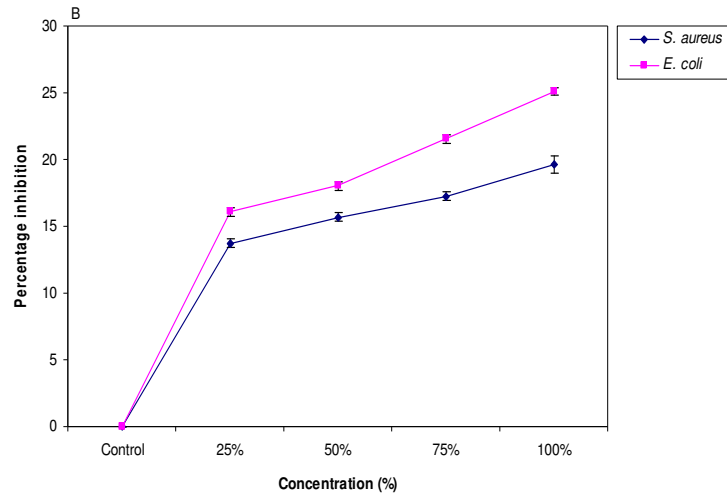


Figure 1

(A) Percentage inhibition of growth of *S. aureus* and *E. coli* against aqueous extract of *C. militaris* and (B) percentage inhibition of growth of *S. aureus* and *E. coli* against methanolic extract of *C. militaris*



Plate 1: (a) *Cordyceps militaris* in its natural habitat. (b) *Cordyceps militaris* showing association with insect host. (c-d) Pure culture of *Cordyceps militaris*.

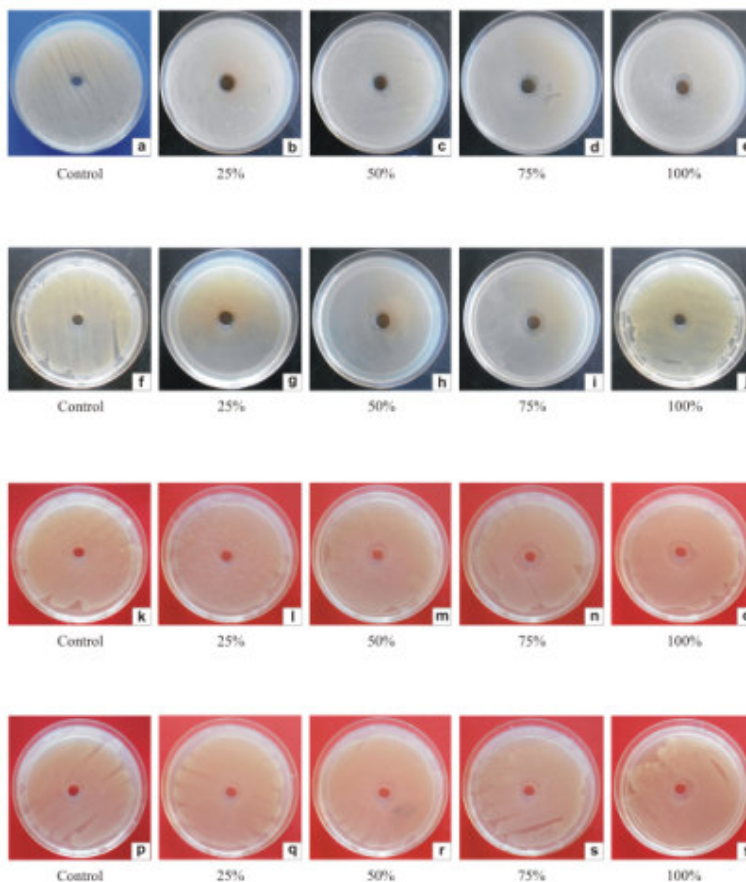


Plate 2: (a-e) Inhibition of growth of *S. aureus* at different concentrations of aqueous extract of *Cordyceps militaris*. (f-j) Inhibition of growth of *E. coli* at different concentrations of aqueous extract of *Cordyceps militaris*. (k-o) Inhibition of growth of *S. aureus* at different concentrations of methanolic extract of *Cordyceps militaris*. (p-t) Inhibition of growth of *E. coli* at different concentrations of methanolic extract of *Cordyceps militaris*.

CONCLUSION

In present study, we have reported the antibacterial activity of aqueous and methanolic extract of *Cordyceps militaris* against *S.aureus* and *E.coli*. So, there is a need for further studies to isolate and characterize the bioactive compounds present in *Cordyceps militaris* and these metabolites can be used to develop effective drugs against these human pathogenic bacterial strains.

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