Research Paper

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FTIR studies and antimicrobial potential of *Cordyceps militaris* (a highly medicinal fungus) against Hospital Pathogens

Microbiology

KEYWORDS : Antimicrobial Activity, Clinical Isolates, Ftir, Wild Medicinal Mushroom

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ABSTRACT

Being Cordyceps militaris (L.)Link recognized as a medicinal mushroom, this work intend to reveal new interesting bioactive molecules that could be isolated from the species, to date there is no defined study on C.militaris from NW Himalayan region. Fourier Transformed InfraRed (FT-IR) spectroscopy revealed the presence of major chemical constituents in the fungus, which was evident from the various peaks in absorption spectra. Hence, the various extracts (ethanol, chloroform, methanol, acetone and aqueous) prepared from the fruiting bodies which contained these active constituents were tested by agar well diffusion method to access their antibacterial potential. Major absorption peaks were of primary amines, alkenes, alcohol and phenols. E.coli was inhibited maximum by ethanolic extracts and showed inhibition zone of 30.58±0.02mm whereas acetone extracts showed maximum inhibition against S.aureus with 36.94±0.05mm inhibition zone at 100% concentration of the extract.The bioactive contents of the mushroom are promising natural antimicrobial agents that can be harnessed as antimicrobial toxicants. In future the C.militaris could provide an alternative method for antibacterial therapy which is a major concern for today mainly in the developing countries like India.

1.Introduction

The continuous use of antibiotics in clinical practice has resulted in the development of multiple resistant microbial strains. This coupled with more recent health trends to adopt a more natural approach to healing has consequently resulted in an increasing need to explore alternative therapies. The treatment of non-healing wounds has also become a global healthcare concern resulting in more laboratory testing, increased hospitalization times and greater treatment options, ultimately resulting in higher financial burdens (Nyasulu et al. 2012). Wild and cultivated mushrooms contain a huge diversity of biomolecules with nutritional (Kalac 2009) and/or medicinal properties (Borchers et al. 2004; Poucheret et al. 2006). *Cordyceps,* a composite of fungus stromata, named as *Cordyceps militaris*, parasitized on the larvae of some species of insects and the dead caterpillar. At present, there are many researches concerning about finding effective ingredients and their pharmacological functions in *Cordyceps* (Committee of National Pharmacopoeia 2000). *C. militaris* has insecticidal, antibacterial and antitumor properties (Paterson and Russel 2008) and polysaccharides that provide anti-inflammatory, antioxidant, antitumor, anti-metastatic, immunomodulatory, hypoglycaemic, steroidogenic and hypolipidaemic activities (Ng and Wang 2005).

Infrared spectroscopy, with the qualities of discriminative, nondestructive, without separating and extracting, fast and effective, can be employed to qualitatively analyze complicated mixture system, to survey all components of the herbs and reflect the overall information of the samples (Sun et al. 2000). Despite the huge diversity of antibacterial compounds, bacterial resistance to first choice antibiotics has been drastically increasing. Diseases that were easily healed are nowadays becoming a serious problem due to the emergent antibiotics resistance (Peres-Bota et al. 2003; World Health Organization report antimicrobial resistance (AMR) 2012). The association between multi-resistant microorganisms and hospital infections certainly highlighted the problem and the urgent need for solutions (Pittet 2005). In 2010, the World Health Organization advised all the countries to implement control procedures for the propagation of drug multi-resistant bacteria, highlighting the risks associated to the absence of alternative therapies against those microorganisms (World Health Organization report antimicrobial resistance (AMR) 2012). Hence, keeping in view the multiple advantages of FTIR and increasing bacterial resistance the present study aims at screening *C. militaris* against two major bacterial pathogens.

2.Materials and Methods

2.1.*Standards and reagents*

The culture media Nutrient Broth was obtained from Himedia. KBr and all the chemicals used were of analytical grade, obtained from Merck. Water was treated in a Milli-Q water purification system (TGI Pure water Systems, Greenville, SC, USA) before use.

2.2.Material

The fresh fruiting bodies of *C. militaris* were collected during the month of August 2013 from Glen forest of District Shimla, (H.P.), India. Just after collection the mature fruiting bodies were cleaned and sun dried. Then these were grounded mechanically into a coarse powder and stored at 4^{o}C for further use.

2.3.*Sample preparation for FTIR*

Sample for FTIR was prepared following (Sharma 1979; Yang et al. 2009). The sample was kept into FT-IR model Nicolet-6700 and FT-IR spectra were taken. Spectra were taken in the region 4000-400 cm^{-1} by employing standard Potassium bromide pellet technique.

2.4.*Extraction for antimicrobial assay*

Aqueous, ethanol, chloroform, acetone and methanolic extracts of experimental fruiting bodies were prepared following (Indian Pharmacopoeia 1996). The dried extracts were collected and stock solution of conc. 40 mg/ml was prepared in the same solvent. Ampicillin was used as reference and its solution was prepared at a concentration of 2mg/ml in sterile distilled water.

2.5.Test Microorganism

The micro-organisms used were clinical isolates from patients hospitalized in various department Hospital center of Indira Gandhi Medical College, Shimla, India. Gram–positive bacteria *Staphylococcus aureus* isolated from wound exudates and Gram–negative bacteria *Escherichia coli* isolated from urine.

2.6.Test assays for antimicrobial activity

Antimicrobial activity of various extracts was tested using agar well diffusion method (Pathania and Sagar 2014). One agar well of 8 mm diameter was prepared with the help of sterilized stainless steel cork borer in each petriplate containing nutrient agar. 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 ± 2^{o}C for 24 hours in 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 and the average values were calculated.

3.Results

3.1.*FTIR Spectra Analysis*: Figure 1 presents the FT-IR spectra of the sample analysed. The strong absorption bands observed at 3321.3 cm^{-1} due to the bonded N–H stretching show the presence of primary amine in this sample. In addition, the bands observed at 2826.0 cm^{-1} in the sample due to C–H symmetric stretch show the presence of alkenes as a common biochemical constituent. The secondary structure of amine due to N–H bending observed between 1652.8 cm^{-1}–1549.3 cm^{-1} (Yang et al. 2009). The bands observed between 1441.7 cm^{-1}–1404.8 cm^{-1} due to bonded C–O / O–H bending (Yang et al. 2012) in alcohols and phenols. The spectral band observed between 1076.8 cm^{-1}–1038.9 cm^{-1} due to SO_3 stretching (Lin et al. 2008) shows the presence of sulfur compounds and were also indicative of β 1-3 glycosidic linkage. The medium bands observed at 776.8 cm^{-1} due to C–H out-of-plane bending (Sharma 1979) show the presence of aromatic compound with substitution of hydrogen. The band observed between 691.6 cm^{-1}–616.4 cm^{-1} due to O–N=O bending (Yang et al. 2009) shows the presence of nitrite. The weak absorption bands observed at 531.0 cm^{-1} due to S–S stretching (Sharma 1979) show the presence of sulphide.

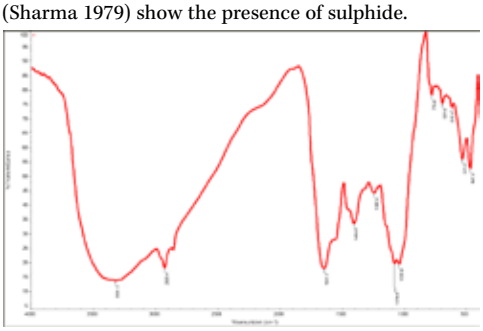

Figure- 1. FTIR spectra of *C. militaris* sample ground with KBr powder and pressed into pellets for FTIR measurement at 4000-400/cm.

3.2. Anti-bacterial assay: Phenolic compounds and β 1-3 glycosidic linkage (indicative of polysaccharides) decipherd from FT-IR spectra provides the evidence of presence of these compounds and these compounds are well known for their antioxidant properties and also have antimicrobial activities (Barros et al. 2007; Ribeiro et al. 2007; Quereshi et al. 2010; Ozen et al. 2011) emerging with potential against multiresistances. Their increasing prevalence is one of the major challenges for the healthcare systems worldwide. Antibiotic resistant infections are associated with a 1.3 to 2 fold increase in mortality compared to antibiotic-susceptible infections (Cosgrove and Carmeli 2004). In the present study, in the range of tested concentrations of the various extracts (10-40mg/ml) there was significant inhibition of both the pathogens (Fig 2 & 3). Control did not inhibit the growth of the bacteria.

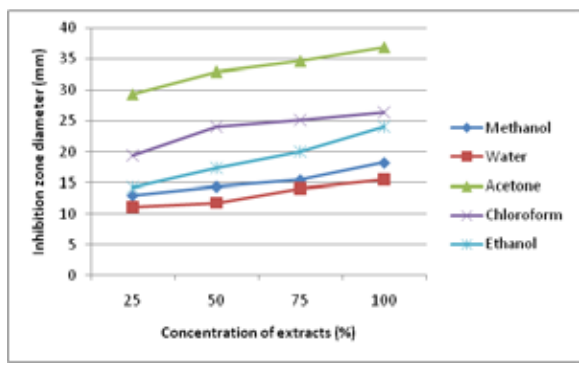

Figure-2. Antibacterial activity of *C.militaris* exracts against *E.coli.*

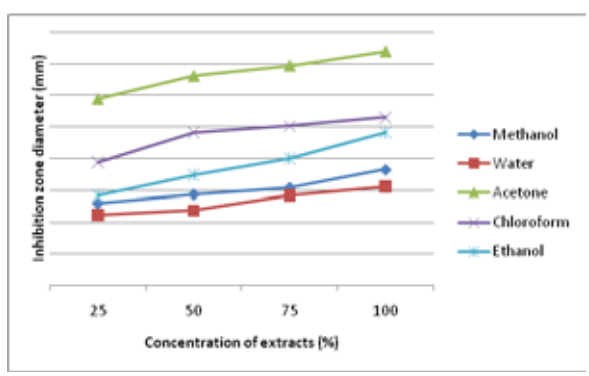

Figure-3. Antibacterial activity of *C.militaris* extracts against *S.aureus.*

However, the reference antibacterial agent ampicillin was effective against both the bacteria and showed inhibition zone of 23.4±0.10 mm and 22.4±0.23mm against *Staphylococcus aureus* and *Escherichia coli* respectively. While at 100% concentration all the extracts were active against both the bacteria. The extracts showed 18.82±0.08, 17.64±0.01, 27.64±0.03, 28.23±0.04 and 30.58±0.02% inhibition against *E.coli.* and 18.23±0.21, 15.52±0.30, 36.94±0.05, 26.47±0.23, 24.11±0.10% inhibition against *S. aureus* respectively for methanol, water, acetone, chloroform and ethanol extracts at 100% concentration of the extract (Table 1& 2).

Table: 1 Antibacterial screening of extracts of *Cordyceps* militaris against *E.coli*

Concentration of extract of *C. militaris* (In %)	Inhibition zone diameter (In mm ± S.E.)				
	Methanol	Water	Acetone	Chloroform	Ethanol
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
25%	11.76±0.02	10.58±0.00	15.29±0.05	14.11±0.03	15.29±0.05
50%	15.29 ±0.04	12.94±0.08	23.52±0.03	17.64±0.05	18.82±0.11
75%	15.65±0.02	14.70±0.13	24.70±0.03	21.17±0.08	22.35±0.04
100%	18.82±0.08	17.64±0.01	27.64±0.03	28.23±0.04	30.58±0.02

Table : 2 Antibacterial screening of extracts of *Cordyceps militaris* against *S. aureus*

Concentration of extract of *C.militaris* (In %)	Inhibition zone diameter (In mm ± S.E.)				
	Methanol	Water	Acetone	Chloroform	Ethanol
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
25%	12.94±0.11	11.05±0.18	29.41±1.21	19.41±0.50	14.17±0.06
50%	14.35±0.18	11.76±0.66	33.05±3.03	24.11±0.23	17.35±0.18
75%	15.41±0.33	14.11±0.14	34.70±0.16	25.17±0.27	20.00±0.14
100%	18.23±0.21	15.52±0.30	36.94±0.05	26.47±0.23	24.11±0.10

4.Discussion

Diseases that were easily healed are nowadays becoming a serious problem due to the emergent antibiotics resistance (World Health Organization report on infectious diseases 2000; Peres-Bota et al. 2003). The association between multi-resistant microorganisms and hospital infections certainly highlighted the problem and the urgent need for solutions (Pittet 2005). The research on mushrooms is extensive and hundreds of species have been demonstrated a broad spectrum of pharmacological activities, including antimicrobial activity. It has been reported that *Cordyceps* contains amino acids, fatty acids, nucleotides, sterols, mannitol, polysaccharides and many other components, in which oleic acid and D-mannitol are in high contents (Liu et al. 2007). FITR spectrum is an important record which gives sufficient information about the structure of a compound. This technique provides a spectrum containing a large number of absorption bands from which a wealth of information can be deciphered about the compound. The absorption of Infra-red radiations causes the various bands in a molecule to stretch and bend with respect to one another (Sharma 1979). Spectroscopy revealed characteristic functional groups of Polysaccharides, peptide and glycosidic linkages hence, suggesting they are PSP complexes and these PSP in future can be used as nutraceutical product for the preparation of functional food. In fact, there is a gap on identification of individual compounds responsible for these antimicrobial properties, and only a few low molecular weight compounds and some peptides and proteins have been described. Further authors are working on the elucidation of their mechanism of action, so as to develop drugs effective against pathogenic microorganisms resistant to conventional treatments.

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6.Conflict of interest: Authors declares no conflict of interest.

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