ZKG INTERNATIONAL ISSN: 2366-1313

Review On Medicinal Mushroom Crude Ethanolic Extracts For Antibacterial Efficacy

S Madhu, Research Scholar, Department of Botany , J.S University, Shikohabad.

Dr BPS Chauhan, Professor ,Supervisor, Department of Botany, J.S University,Shikohabad.

Abstract - The purpose of this research was to assess the antibacterial activity of crude ethanolic extracts that were aged for one year and derived from Cordyceps sinesis, Laricifomes officinalis, Oudem ansiellamucida, and Coprinus comatus. Extracts were tested for their ability to inhibit the growth of Bacillus thuringiensis and Staphylococcus aureus, both of which are examples of Gram-positive bacteria, as well as Klebsiella pneumoniae and Enterobacter aerogenes, both of which are examples of Gram-negative bacteria. The results of these tests were analyzed in this research. For the purpose of determining the antibacterial activity of the extracts, the disk diffusion technique and the microbroth dilution method, both of which adhere to the recommendations established by the European Committee on antibacterial Susceptibility Testing (EUCAST), were used. The studies were carried out using microplates with 96 separate wells. The Czech Collection of Microorganisms was the source of the microorganisms that were used. After converting the data to a binary system, the results of the Probit analysis were applied to the values that were acquired by subtracting the absorbance readings from both before and after the experiment. In none of the ethanolic extracts of macromycetes did we find evidence of the bacteria's ability to inhibit the growth of other microbes. Using the MIC approach, we evaluated the antimicrobial activity of Oudem ansiellamucida, Cordyceps sinesis, and Coprinus comatus extracts. The findings suggested that the extracts were effective within the tested range, which indicated that the extracts were antimicrobially active. The extract that was obtained from Laricifomes officinalis had, on the other hand, the most remarkable antibacterial effectiveness, as evaluated using disc diffusion methods. In a similar vein, it will be essential to conduct an inquiry into the antibacterial characteristics of these mushrooms in a more in-depth manner.

Keywords: Antimicrobial activity, macromycetes ethanolic extracts, MIC, edible mushrooms

1. INTRODUCTION

Since the beginning of time, nature has been used as a valuable reservoir for a wide variety of different medicinal compounds. Over the course of the last few decades, there has been a steadily increasing level of concern regarding the emergence of bacteria that are resistant to antibiotic treatment. In order to protect themselves against antimicrobial agents, bacterial pathogens have evolved a variety of defensive methods. Therefore, there is a growing demand for the investigation of novel and more effective agents to supplement or replace antibiotic therapy (Butler et al., 2004; Lam et al., 2007). This is due to the fact that antibiotics are becoming increasingly ineffective. Macromycetes, which are categorized as higher fungus, are rich sources of physiologically active chemicals that display a diverse range of chemical structures. According to research conducted by Alves et al. (2012), mushrooms have the potential to serve as a source for the development of effective antibacterial drugs. The presence of chemicals with antibacterial properties is essential to the continued existence of mushrooms in their natural environment. As shown by Lindequist et al. (1990), the process of isolating antimicrobial chemicals from a variety of mushrooms and determining whether or not they have any use in human medicine is a well-established occurrence. India (Seena et al., 2003; Quereshi et al., 2010) and China (Gao et al., 2005) have both been the focus of a number of research efforts that have investigated the antibacterial properties of different fungal extracts. In this nation, the practice of using medicinal fungi dates back many years and is considered to be a time-honored custom. Ganoderma lucidum is a well-known traditional medicinal fungus that is often used as a functional food and in preventative medications. It has been used for medical purposes for a long time. According to Sullivan et al. (2005) and Pala et al. (2011), it is generally eaten in the form of extracts and has a considerable market all over the world. To this day, the only antibiotics that are suitable for use in a commercial setting are those that are produced from very small fungi (Lindequist et al., 2005).

The primary objective of this research is to investigate the antibacterial capabilities of extracts obtained from medicinal mushrooms that have been aged for one year. Cordyceps sinesis, Laricifomes officinalis, Oudemansiellamucida, and Coprinus comatus are the species of mushroom that are in concern here. The purpose of this research is to determine whether or not these extracts are effective against a wide variety of Gram-positive and Gram-negative bacteria.

2. MATERIALSANDMETHODS

➢ Fungi materials

The materials utilized in this experiment are derived from Solid State Fermentation (SSF) of Cordyceps sinesis and the basidiocarps of Laricifomes officinalis, Oudemansiellamucida, and Coprinus comatus fungi. The dried fungi obtained through solid-state fermentation (SSF) and the fruiting bodies were procured from Mykoforest, a company based in Slovakia. The identification of certain fungi was carried out by Martin Rajtar, an expert from Mykoforest in Slovakia. The fungi were subjected to desiccation at ambient conditions in the absence of light.

➢ Testmicroorganisms

The present investigation examines the impacts of four distinct strains of microorganisms. Klebsiella pneumoniae CCM 2318 and Enterobacter aerogenes CCM 2531 are instances of Gram-negative and Gram-positive bacteria,

correspondingly. Staphylococcus aureus subsp. aureus ser. a5 CCM 2461 and Bacillus thiringiensis CCM 19 are Gram-positive bacterial species. All tested strains were obtained from the Czech Collection of Microorganisms, where they were originally collected. The bacterial suspensions were cultured in nutritional broth (Imuna, Slovakia) under controlled conditions. The temperature for the majority of the bacterial suspensions was set at 37 degrees Celsius, while Bacillus thiringiensis was cultured at a temperature of 30 degrees Celsius.

- **Preparationoffungalextracts**

After the fungal materials had been dried, they were ground into a powder and their weight was accurately recorded as 10 grams. After that, each of the samples was submerged in 100 mL of high-grade ethanol (99.5%, Sigma, Germany) and allowed to soak for a period of twelve months at room temperature and in an environment with a low level of light exposure. What are the considerations that led to the decision to make the term of one year? The major purpose of this study was to evaluate the antibacterial activity after the product had been stored for a significant amount of time. In order to stop the active components from degrading over time, it was necessary to take precautions against being exposed to sunlight. Following that, the ethanol in the ethanolic fungal extracts was removed by evaporating the mixture at a temperature of 40 degrees Celsius while maintaining a reduced pressure. This was done with a rotary evaporator manufactured by Bibby Scientific Limited in the United Kingdom (Stuart RE300DB) and a vacuum pump manufactured by KNF in Germany (KNF N838.1.2KT.45.18). During the antimicrobial test, the crude fungal extracts were first dissolved in dimethyl sulfoxide (DMSO) from Penta, Czech Republic, in order to reach a stock solution concentration of 102.4 mg/mL. This was done so that the results of the test could be interpreted. Before being used, the fungal extract stock solutions were kept in a refrigeration unit at a temperature of -16 degrees Celsius until it was time to use them.

The process of preparing discs and conducting the disc diffusion method.

Synchronously with evaporation of ethanol from mushroom extract blank discs (Oxoid, UK) were added to extracts and impregnated with extracts. Discs stayed in extracts until evaporated completely. Obtained impregnated discs served as pre-determination experiment for detection of antimicrobial activity. Concentration of extracts in discs were unknown. Impregnated discs were used for disc diffusion methodology, which was done on Mueller-Hinton agar (Biolife, Italy) at 37 °C for three bacteria, expect Bacillus thuringiensis (30°C) during 16- 20 hours. Bacterial inoculum in physiological solution at the final density of 0,5 McF° was spread out on the agar surface evenly. Impregnated discs were stacked on to the agar surface evenly with adequate spacing. Inhibition zones were read in millimeter.

3. ANTIMICROBIAL ASSAY

The term "minimum inhibitory concentration" (MIC) refers to the lowest concentration of a specific sample that is able to stop the outward manifestation of microbial growth. The formulation of a fungal extract solution was accomplished by dissolving the extracts in DMSO, which led to a concentration of 4096 g/mL in the final product. For the purpose of determining the minimum inhibitory concentrations (MICs) of bacteria in Mueller Hinton broth (Biolife, Italy), the microbroth dilution method was used. This technique is one of the ones that has been suggested by the Clinical and Laboratory Standards

Institute (CLSI, 2009). In conclusion, a series of dilutions by a factor of two were performed in order to produce DMSO fungal extract solutions that had a final concentration that varied from 2 to 4096 g/mL. After that, a microbial suspension with a final density of 0.5 McF° was poured into each well, and the experiment was stopped. After 16 to 20 hours of incubation at a temperature of 30 degrees Celsius for Bacillus thuringiensis and 37 degrees Celsius for the other three bacterial strains. Inhibition of microbial growth was determined by measuring well absorbance at 590 nm using a shaker-equipped Biotek EL808 absorbance microplate reader (Biotek Instruments, USA). This was done in order to get an accurate reading. Before beginning and after finishing the experiment, the 96-microwell plates were analyzed for their condition. Analyzing the differences that were found between the two measures allowed for an evaluation of the growth. Absorbance values of 0.05 were used to investigate the possibility of mistake in the measuring process. The use of wells that did not include any fungal extracts was crucial in the establishment of positive growth controls. The use of unadulterated dimethyl sulfoxide (DMSO) as the study's negative control allowed for more accurate results. In order to improve the accuracy of the calculated minimum inhibitory concentrations of the fungal extracts used in the experiment, the study was carried out using eight separate duplicates.

- **Statisticalanalysis**

We were able to express the differences in absorbance among the measurements as a string of binary digits by making use of the absorbance values that were recorded before to and after the analysis. These numbers were given very specific concentrations to go along with them. In the current investigation, a method was used that had been conceived only with the objective of carrying out this particular experiment in mind. If the absorbance value was less than 0.05, an inhibitory effect was awarded the value 1, and if the absorbance value was more than 0.05, a stimulant effect was assigned the value 0. A value of 0 was assigned to show that there was either no effect or a stimulant effect. The Probit analysis included in Statgraphic software was used for the statistical assessment.

4. RESULTSANDDISCUSSION

- **Disk diffusion method**

The outcomes of the disc diffusion tests suggested that the extracts obtained from Oudemansiellamucida and Laricifomesofficilnalis have antibacterial characteristics. Several researchers have reported their findings (Anke et al., 1979; Anke et al., 1990; Florianowicz et al., 1999) indicating that extracts derived from Oudemansiellamucida possess selective antifungal properties. The researchers carried out an experiment in which they discovered that the principal chemicals, strobilurins and oudemansins, inhibited the development of many yeasts, including Candida albicans, Candida glabrata, Candida krusei, and Candida tropicalis. As shown in Figure 1D, particularly in sample 9, the results of the current experiment revealed an inhibitory impact against Staphylococcus aureus. Specifically, this effect was shown in sample 9. In the instance of Staphylococcus aureus, exposure to Oudemansiellamucida extract resulted in the observation of an inhibitory zone of 12 millimeters in diameter. According to the results of this experiment, extracts of Laricifomes officinalis displayed inhibitory action against all of the bacterial strains that were used in the research. The extract of Laricifomes officinalis showed inhibitory zones of 27 millimeters against Staphylococcus

aureus, 13 millimeters against Bacillus thuringiensis, 20 millimeters against Klebsiella pneumoniae, and 20 millimeters against Enterobacter aerogenes. According to the findings of the inquiry, the other extracts of macromycetes used in the study have no antibacterial capabilities whatsoever. In a study that was carried out by Demir and Yamac (2008), the researchers investigated the antibacterial potential of an extract of Coprinus comatus basidiocarp that had been dissolved in a variety of different solutions. The extract was tested on four different strains of bacteria: Staphylococcus aureus, Enterococcus faecium, Proteus vulgaris, and Candida glabrata. The submerged mycelium and exopolysaccharides from Coprinus comatus were put through tests; however, the detected activity was not nearly as comprehensive as that which was seen in an earlier test using basidiocarps. Figure 1 presents an illustration of the inhibition zones.

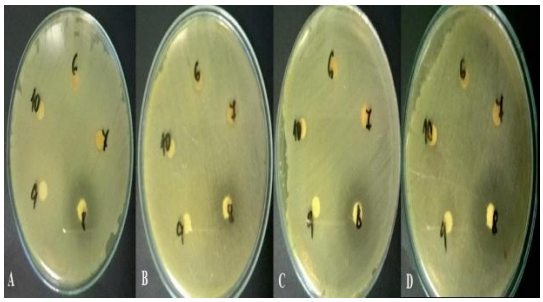

Figure 1 Inhibition zones formed around fungal extract discs (A) Klebsiella pneumoniae, (B) Bacillus thuringiensis, (C) Enterobacter aerogenes,(D) Staphylococcus aureus, (6) Coprinus comatus, (7) Cordyceps sinensis, (8) Laricifomes officinalis, (9) Oudemansiellamucida and (10) not presented extract in this study

- **Minimalinhibitionconcentration**

Table 2 provides a summary of the antimicrobial activity of four ethanolic fungal extracts obtained from Cordyceps sinesis, Laricifomes officinalis, Oudem ansiellamucida, and Coprinus comatus against four different bacterial strains. The antimicrobial activity is indicated in micrograms per milliliter (g/mL). An evaluation of the effectiveness of a fungal extract obtained from Coprinus comatus against Enterobacter aerogenes yielded a minimum inhibitory concentration (MIC) 50 value of 2048 ug/mL. This value was determined by the evaluation. In addition to this, the extract of Oudem ansiellamucida had an inhibitory effect against the bacterium that was examined. According to the results, the Laricifomes officinalis plant displayed growth-inhibiting activities against all of the bacterial strains that were examined using the disk diffusion technique. The minimum inhibition concentration approach, on the other hand, did not exhibit any inhibitory action at any concentration within the range that was evaluated. Investigations on the antibacterial capabilities of Cordyceps sinensis have been carried out throughout a number of different research projects. According to Kniefel et al. (1977) and Ahn et al. (2000), the primary compounds present in Cordyceps sinensis, specifically cordycepin, have been discovered to exhibit significant effects against a variety of microorganisms. These microorganisms include Clostridium perfringens and C. paraputrificum, as well as Bifidobacterium spp. and Lactobacillus spp. The antibacterial capabilities of higher fungi have been the subject of a great deal of research, as shown by the work of Yoon et al. (1994), Rosa et al. (2003), Poucheret et al. (2006), Molitoris (1994), and Lindequist et al. (2005), to name just a few of the researchers who have contributed to this field. This is owing to the fact that mushrooms need chemicals with antibacterial and antifungal properties in order to flourish in their natural environment. It is a well-documented fact that antimicrobial chemicals can be isolated from a variety

of mushrooms, and that these compounds may have potential use in human medicine (Lindequist et al., 1990). To this day, the only substances that have been made available for use in commercial settings are those that are derived from extremely small fungi and antibiotics (Lindequist et al., 2005).

5. CONCLUSIONS

In conclusion, it is possible to declare that the ethanolic fungal extracts generated from Cordyceps sinesis, Laricifomes officinalis, Oudemansiellamucida, and Coprinus comatus demonstrated strong antibacterial activity at increased concentrations. These extracts were tested against bacteria, yeast, and mold. The experiment produced surprising findings, as the extract obtained from Laricifomes officinalis displayed the most effective antibacterial action as assessed using disk diffusion technique, rather than MIC methodology. This result was established by comparing the amount of microbial growth inhibited by the extract to the minimum inhibitory concentration (MIC). In order to have a better knowledge of the antibacterial characteristics possessed by macro fungal extracts, more study is required. This necessitates the carrying out of more investigations and tests, as well as increasing the breadth of the concentration ranges that are investigated.

REFERENCES

1. Alves,M.J.,Ferreira,I.C.F.R.,Dias, J.,Teixeira,V.,Martins,A.,Pintado, M.2012.Areviewonantimicrobiala ctivityofmushrooms(Basidiomyce tes)extracts and isolated compounds. Planta Med., 78, 1707-1718.http://dx.doi.org/10.1055/s-0032-1315370
2. Balakumar,R.,Sivaprakasam,E.,K avitha,D.,Sridhar,S.,Kumar,J.S.20 11.Antibacterialandantifungalacti vityoffruitbodiesofPhellinusmushr oomextract.InternationalJ.Biosc.,1 (3),72-77.
3. Butler,M.S. 2004.Theroleofnaturalproductche mistryindrugdiscovery.Journal of NaturalProducts, 67, 2141-2153.http://dx.doi.org/10.1021/np040106y
4. ClinicalandLaboratoryStandardIn stitute.Performancestandardforant imicrobial susceptibility testing;informational supplementM100-S21. 21thed. Wayne,PA:CLSI;2009.
5. Demir,M.S.,&Yamac,M.(2008).A ntimicrobialactivitiesofbasidiocar p,submergedmyceliumandexopol ysaccharideofsomenative Basidiomycetesstrains. Journal of Applied Biological Sciences, 2(3), 89-93.
6. Gao,Y.,Tang,W.,Gao,H.,Chan,E., Lan,J.,Li,X.,Zhou,S.2005.Antimic robialactivityofmedicalmushroom s*Ganoderma*.FoodRev.Int.,21,211 -229.http://dx.doi.org/10.1081/fri-200051893
7. Heleno,S.A.,Ferreira,I.C.F.R.,Este ves,A.P.,Ćirić,A.,Glamočlija,J.,M artins,A.,Soković,M.,Queiroz,M.J. R.P.2013.Antimicorbialanddemel anizingactivity ofGanoderma lucidum extract, p-hydroxybenzoic and cinnamic acidsandtheirsyntheticacetylatedgl ucuronidemethylesters.FoodandC hemicalToxicology,58,95-100.http://dx.doi.org/10.1016/j.fct.2013.04.025
8. Kavitha,D.,Balakumar,R.,Sivapra kasam,E.,Sridhar,S.,Suresh,J.2011. Antibacterialandantifungalpotenti aloffruitbodyextractsfromdaldinia concentrica (bolton) cesati& de notaris. International journal of

pharmaceuticalsciencesandresearch,2(9),2376-2379.

9. Nikolovska-Nedelkoska, D., Atanasova-Pančevska, N., Amedi, H., Veleska, D., Ivanova, E., Karadelev, M., Kungulovski, D. 2013. Screening of antibacterial andantifungal activities of selected Macedonian wild mushrooms. Zbornik Matice srpske za prirodne nauke, (124), 333-340.http://dx.doi.org/10.2298/zmspn1324333n
10. Rosa,L.H., Machado,K.M.G.,Jacob, C.C.,Capelari, M.,Rosa, C.A.,&Zani,C.L.(2003).Screening ofBrazilianbasidiomycetesforantimicrobialactivity.*Memórias do Instituto Oswaldo Cruz*,*98*(7), 967-974.http://dx.doi.org/10.1590/s0074-02762003000700019
11. Sheena, N., Ajith, T. A., Mathew, A.T., Janardhanan, K.K. 2003. Antibacterialactivityofthreemacrofungi,Ganodermalucidum,NavesporusfloccosaandPhellinusrimosusoccuringinSouthIndia.Pharm.Biol., 41,564-567.http://dx.doi.org/10.1080/13880200390501226
12. Suay, I., Arenal, F., Asensio, F.J., Basilio, A., Cabello, A.M., Díez, M.T., García,J.B.,GonzálezdelVal,A.,Gorrochategui,J.,Hermández,P.,Peláez,F.,Vincente, M.F. 2000. Screening of basidiomycetes for antimicrobial activities.AntoinevanLeeuwenhoek JournalofMicrobiology, 78(2),129-140.