Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8):307-312

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antimicrobial activity of macrofungi of Rajouri Dist. (J & K), India

Nidhi Anand*[1,2], P. N. Chowdhry[2] and Abhishek Mathur[2]

[1]Mewar University, Chittorgarh, Rajasthan, India
[2]National Centre of Fungal Taxonomy (NCFT), New Delhi, India

---

ABSTRACT

In the present investigation, antimicrobial activity and phytochemical screening was evaluated for the three newly reported macro fungal species from Rajouri District, J&K, India. The hydro-alcoholic extracts of the dried mushroom samples viz. Krombholziella oxydabilis (A), Daldenia concentrica (B) and Innotus radiate (C) at 200 mg/ml were evaluated against bacterial and fungal organisms viz. Pseudomonas aeruginosa, Bacillus licheniformis, Micrococcus luteus, Aspergillus niger and Cladosporium herbarum. The results showed that mushroom species viz. Krombholziella oxydabilis and Innotus radiate can be utilized as a suitable antimicrobial agent while mushroom species, Daldenia concentrica doesn't possessed antimicrobial activity against any of the fungal and bacterial strains studied.

Keywords: Macro fungi, antimicrobial activity, phytochemical screening, active molecules.

INTRODUCTION

We have made tremendous progress in technology and human medicine; But still we are threaten by various new strains of bacteria, fungi and virus causing diseases especially in the developing countries [1]. The unavailability of medicines in developing nations and in the extensive drug resistance of microorganisms has a large impact on human health [2]. So there is earnest need of research for investigation of new antimicrobial substances .Natural products have immense potential of containing antimicrobial agents for various microorganisms cause of infectious diseases [3]. Macro fungi have a potential of using both as nutritive and medicinal food stuff [4-7]. It is not surprising that mushrooms are a source of many biologically active compounds. Mushrooms manage to grow in darkness and dampness in highly competitive environments and protect themselves from hordes of attacking microbes by developing natural protective substances [8]. Researchers isolated and identified some compounds, originating from Macro fungi show other medicinal properties, such as immuno-modulatory, liver protective, antifibrotic, anti-inflammatory, anti-diabetic, antiviral and antimicrobial activities [9-13]. The mushroom species are valuable source of pharmacological agents. Different phytochemical constituents are present within it apart from fibers and sugars. It is not necessary that, the mushroom species which are not edible will not be a pharmacological agent. Mushrooms producing antibiotics are undoubtedly numerous, but so far, they have not been sufficiently studied. Much work has been carried out on the antimicrobial activities of micro fungi but macro fungi have not been adequately explored. Therefore, the study was undertaken to determine the antimicrobial activity of three newly reported Macrofungal species from Rajouri District, J&K, India viz. *Krombholziella oxydabilis* (A), *Daldenia concentrica* (B) and *Innotus radiate* (C).

EXPERIMENTAL SECTION

Preparation of extracts of mushroom samples

The mushroom samples viz. *Krombholziella oxydabilis* (A), *Daldenia concentrica* (B) and *Innotus radiatus* (C) were washed with distilled water, sterilized in autoclave at 105^0C for 15 minutes, dried under shade and pulverized. The method of Alade and Irobi (1993) was adopted for preparation of solvent extracts with little modifications. Briefly, 20 g portions of each of the powdered material of mushroom samples were soaked separately in 50% (v/v) ethyl alcohol for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) The filtrates obtained were concentrated in vacuo using water bath at 30 ^{0}C .

Determination of Antimicrobial activity

Culture Media

For antibacterial test, Soyabean casein digest agar/broth and Potato dextrose agar/broth of Hi Media Pvt. Bombay, India was used for antifungal test.

Inoculum

The bacteria were inoculated into Soyabean casein digest broth and incubated at 37 ^{0}C for 18 h and suspension was checked to provide approximately, 10^5 CFU/ml. The same procedure was done for fungal strains and there strains were inoculated into Potato dextrose broth but the fungal broth cultures were incubated at 48-72 h.

Microorganisms used

The pure cultures of test bacterial and fungal organisms viz. *Pseudomonas aeruginosa* ATCC 25619 were obtained from Institute of Microbial Technology, Chandigarh, India while *Bacillus licheniformis* (Local isolated strain), *Micrococcus luteus* (Local isolated strain), *Aspergillus niger* (Local isolated culture) and *Cladosporium herbarum* procured from Dept. of Microbiology, Shoolini University, Solan (H.P), India.

Determination of diameter of zone of inhibition by well diffusion method

The agar well diffusion method (Perez *et al*., 1993) was modified. Soyabean casein digest agar medium (SCDM) was used for bacterial cultures. The culture medium was inoculated with the bacteria separately suspended in nutrient broth. Potato dextrose agar/broth was used for fungal cultures. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with mushroom extracts and solvent blanks. Standard antibiotic (Erythromycin, 1 mg/ml) was simultaneously used as the positive control. The plates were then incubated at 37 ^{0}C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. The same procedure as that for determination of antibacterial property was adopted and then after the diameter of zone of inhibition was observed after 48-72 h. Fucanazole (1mg/ml) was used as standard for determination of antifungal activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

Phytochemical screening of the extract

The portion of the extract was subjected to the phytochemical screening using the method adopted by Trease and Evans [15] and Harbourne [16]. Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars and cardiac glycosides [17].

- **Test for alkaloids**

The 0.5 g of the extract was dissolved in 5 ml of 1% HCl and was kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Dragendroff's reagent Turbidity or precipitation was taken as indicator for the presence of alkaloids.

- **Test for Tannins**

About 0.5 g of the sample was dissolved in 10 ml of boiling water and was filtered. Few ml of 6% FeCl3 was added to the filtrate. Deep green colour appeared confirmed the presence of tannins [15]

- **Test for Flavanoids**

About 0.2 gm of the extract was dissolved in methanol and heated for some time. A chip of Mg metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was indicator of the flavanoids.

- **Test for Saponin**

About 0.5 g of the extract was stirred with water in the test tube. Frothing persists on warming was taken as a evidence for the presence of saponin.

- **Test for Steroids**

Salkowaski method was adopted for the detection of steroids. About 0.5 g of extract was dissolved in 3 ml of chloroform and filtered. To the filtrate, conc. $H_2 SO_4$ was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring [18].

- **Test for Cardiac glycoside**

About 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% FeCl3. This was under laid with conc. $H_2 SO_4$. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

- **Test for reducing Sugars**

1 ml each of Fehling's solutions, I and II was added to 2 ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

RESULTS AND DISCUSSION

Antimicrobial activity

It was found that that the hydro-alcoholic extracts of mushroom samples viz. *Krombholziella oxydabilis* and *Innotus radiatus* showed significant antimicrobial activity at 200 mg/ml against the bacterial and fungal strains. It was found that, mushroom species, *Daldenia concentrica* doesn't possessed antimicrobial activity against any of the fungal and bacterial strains studied. Amongst the 3 mushroom species studied, *Innotus radiate* extracts had most prominent antimicrobial activity. It was found that *Aspergillus niger* was the most resistant fungal strain against the attack of any of the mushroom extracts. The results were found to be in reference with the positive control viz. antifungal (Flucanazole, 1mg/ml) and antibacterial (Tetracycline,1mg/ml). The results are shown in **Table 1** and **Figure 1 (a-c)**.

Table 1: Antimicrobial activity of hydro-alcoholic extracts (50% v/v) against pathogenic microbial strains

Mushroom samples	Diameter of zone of inhibition (mm) against microbial strains					
	Cladosporium herbarum	***Aspergillus niger***	***Bacillus licheniformis***	***Pseudomonas aeruginosa***	***Lactobacillus sp.***	***Micrococcus luteus***
A (200 mg/ml)	8.0	NA	10.0	10.0	12.0	10.0
B (200 mg/ml)	NA	NA	NA	NA	NA	NA
C (200 mg/ml)	10.0	NA	8.0	12.0	15.0	12.0
Fucanazole (1 mg/ml)	23.0	24.0	NT	NT	NT	NT
Tetracycline (1 mg/ml)	NT	NT	23.0	21.0	21.0	18.0

**Mushroom samples: A, Krombholziella oxydabilis; B, Daldenia concentrica; C, Innotus radiatus*
**NT, Not tested; NA, No activity*

Figure 1 (a): Hydro-alcoholic solvent extraction of mushroom samples

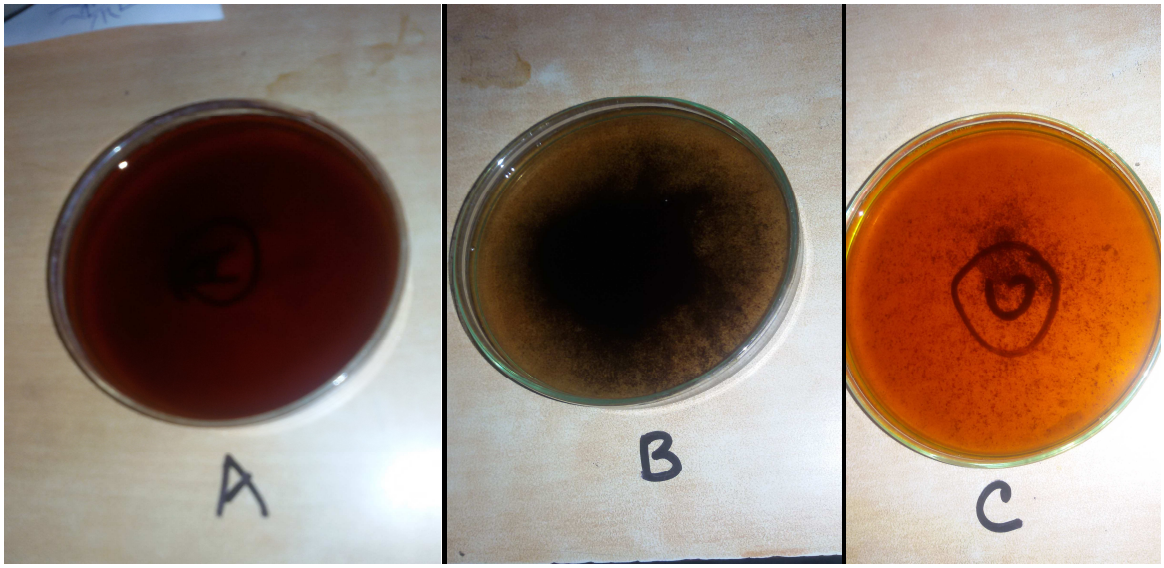

Figure 1 (b): Hydro-alcoholic solvent extraction of mushroom samples

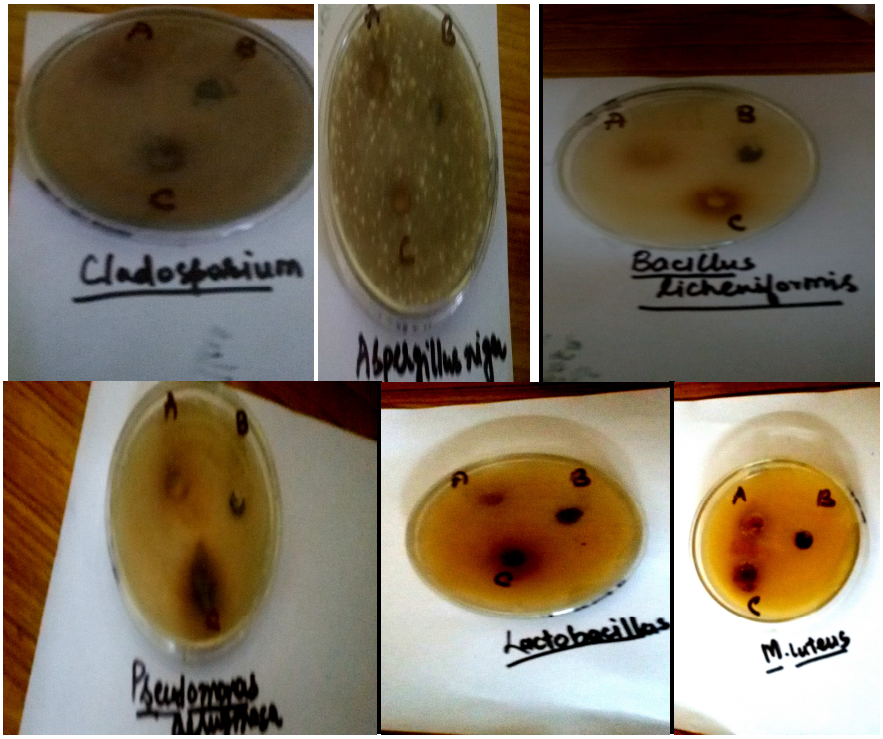

Figure 1 (c): Antimicrobial activity of hydro-alcoholic extracts of mushrooms

Phytochemical Screening

In the present investigation, it was observed that the mushroom samples viz. *Krombholziella oxydabilis* and *Innotus radiate* were having most of the phytochemicals in comparison to *Daldenia concentrica*. The results are shown in **Table 2**.

Table 2: Phytochemical screening of hydro-alcoholic extracts of mushroom samples

Mushroom samples	Phytochemical constituents						
	Alkaloids	Flavanoids	Tannins	Steroids	Saponin	Glycosides	Reducing sugars
Krombholziella oxydabilis	+	+	+	+	+	+	+
Daldenia concentrica	-	-	-	-	+	-	+
Innotus radiates	+	+	+	+	+	+	+

**+, present; -, absent*

CONCLUSION

The present study suggests that, the mushroom species are valuable source of pharmacological agents. Different phytochemical constituents are present within it apart from fibers and sugars. It is not necessary that, the mushroom species which are not edible will not be a pharmacological agent. The present of different active components within the mushrooms constitute its integrity as a suitable pharmacological agent. Thus as per the present study, mushroom species viz. *Krombholziella oxydabilis* and *Innotus radiates* can be utilized as a suitable antimicrobial agent. The studies can be performed further in order to isolate the active principles responsible for antimicrobial activity.

REFERENCES

[1] P Cos, AJ Vlietinck, D Vanden Berghe, L Maes. *Journal of Ethnopharmacology*, **2006**, 106, 290-302, 2006.
[2] IN Okeke, R Laxmaninarayan, ZA Bhutta, AG Duse, P Jenkins, TF Brien, A Pablos-Mendez, KP Klugman. *Lancet Infectious Diseases*, **2005**, 5, 481-493.
[3] J Clardy, C Walsh. *Nature*, **2004**, 432, 829-837.
[4] A Imtiaj, TS Lee. *World Journal of Agricultural Sciences*, **2007**, 3, 316-321.

[5] M Bonatti, P Karnopp, HM Soares, SA Furlan. *Food Chemistry*, **2004**, 425-428.
[6] LM Cheung, PCK Cheung. *Food Chemistry*, **2005**, 89, 403-409.
[7] D. Agrahar-Murugkar, G. Subbulakshmi. *Food Chemistry*, **2005**, 89, 599-603.
[8] N Anand and PN Chowdhry. *Int J Pharm Sci Res*, **2013**, 4(5), 1743-1746.
[9] SP Wasser, AL Weis. *International Journal of Medicinal Mushrooms*, **1999a**, 1, 31-62.
[10] SP Wasser, AL Weis. *Critical Reviews of in Immunology*, **1999b**, 19, 65-96.
[11] N Gunde-Cimerman. *International Journal of Medicinal Mushrooms*, **1999**, 1, 69-70.
[12] VEC Ooi. Medicinally important fungi. In: Van Griensven (Ed.) Science and Cultivation of Edible Fungi, Balkerna, Rotterdam, **2000**, pp. 41-51.
[13] B. Dülger, F. Şen, F. Gücin. *Turkish Journal of Biology*, **1999**, 23, 127-133.
[14] PI Alade, ON Irobi. *Journal of Ethnopharmacology*, **1993**, 39, 171-174.
[15] GE Trease, WC Evans. Pharmacogonasy, 14th Edition, Brown Publication, **1989**.
[16] JB Harborne. Phytochemical method, 3rd Edition, Chapman and Hall, London, **1993**, pp.135-203.
[17] AO Sofowora. Traditional Medicine and Medicinal Plants in Africa. 2nd Ed., University of Life Press, **1993**, pp. 320.
[18] A. Agarwal, IJ Singhvi, D Bele, K Sharma, SK Gupta, G Karwani, M Kumawat M. *International J. Pharmacy & Technology*, **2011**, 3(2), 2480-2486.