The Journal of Phytopharmacology 2016; 5(2): 79-82
Online at: www.phytopharmajournal.com

The Journal of Phytopharmacology
(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2230-480X
JPHYTO 2016; 5(2): 79-82
March- April
© 2016, All rights reserved

Debendra Nath Roy
Department of Pharmacy, Jessore University of Science and Technology, Jessore- 7408, Bangladesh

A. K. Azad
Department of Pharmacy, Bangladesh University, Mohammadpur, Dhaka-1207, Bangladesh

Farzana Sultana
Department of Pharmacy, Jessore University of Science and Technology, Jessore- 7408, Bangladesh

A.S.M. Anisuzzaman
Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

Correspondence:
Debendra Nath Roy
Department of Pharmacy, Jessore University of Science and Technology, Jessore- 7408, Bangladesh
Email: dn.roy[at]just.edu.bd

In-vitro antimicrobial activity of ethyl acetate extract of two common edible mushrooms

Debendra Nath Roy*, A. K. Azad, Farzana Sultana, A.S.M. Anisuzzaman

ABSTRACT

Ethyl acetate extracts of the reishi (*Ganoderma lucidum*) and oyster (*Pleurotus ostreatus*) mushrooms were tested for their in vitro growth inhibitory activity against a panel of microorganisms of reference strains. Anti-microbial effects of both mushrooms were monitored in the dose of 400 micro gm /disc by disc diffusion method using five Gram-positive bacteria, five Gram-negative bacteria and three fungi as well. Present study showed that ethyl acetate extract of *Pleurotus ostreatus* has moderate and *Ganoderma lucidum* has only mild antibacterial effect in comparison with standard Kanamycin (30 µgm. /disc). However, both mushrooms had no antifungal activity on the experimental fungi compared with standard drug Nystatin at the dose of 30 µgm. /disc.

Keywords: Gram-positive, Gram-negative, antibacterial, antifungal.

INTRODUCTION

The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community [1-2]. Mushrooms have long been valued as delicious and nutritional medicinal foods in many countries, which are appreciated, not only for texture and flavour but also for their chemical and nutritional characteristics. On a dry weight basis, they are considered to be good sources of digestible proteins (10–40%), carbohydrates (3–21%) and dietary fiber (3–35%). Mushrooms contain all the essential amino acids and are limiting in the sulfur containing amino acids, cysteine and methionine [3]. Mushrooms are also considered as functional foods because they elicit their positive effect on human being in several ways [4].

Oyster has been explored to combat simple and multiple drug resistant isolates of *Eschrichia coli*, *Staphylococcus epidermidis*, *S. aureus* [5] and species of *Candida* [6], *Streptococcus*, *Enterococcus* [7-10]. Methanolic extracts of *Pleurotus* species demonstrated an inhibition in growth of *Bacillus megaterium*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *C. albicans*, *C. glabrata*, and species of *Trichophyton* and *Epidermophyton* to different degrees that was lower with respect to two antifungal agents: Streptomycin and Nystatin [5]. Antimicrobial and antifungal activity of *OM* depended upon the nature of the solvent, ether extract were more active against Gram negative bacteria as compared to acetone extract [11]. Ether and acetone extracts of *OM* was effective against *B. subtilis*, *E. coli* and *S. cerevisiae*.

Ganoderma lucidum (commonly known as Reishi mushroom) is one of the most famous traditional medicinal mushrooms. Various extracts have been found to be equally effective when compared with gentamycin sulphate, the acetone extract being the most effective. This mushroom had moderate inhibition against *Bacillus subtilis* and *Staphylococcus aureus* for any extract [12], but in the study reported by Sheena et al. [13] its methanolic extract showed poor antimicrobial activity. Other authors described the capacity of the aqueous extract to inhibit 15 types of Gram-positive and Gram-negative bacteria, with the highest inhibition exhibited against *Micrococcus luteus* [14]. Extracts of *Ganoderma* species havebeen very useful in the treatment of various human ailments right from microbial infections to viral infections including HIV due to its immune potentiation and immune modulatory properties; treatment of malignancies such as lung cancer, cardiac failure [15- 17].

MATERIALS AND METHODS

Collection of the materials

The oyster (*Pleurotus ostreatus*) and Reishi (*Ganoderma lucidum*) mushroom were selected for this investigation. These were collected and identified from mushroom development and extension centre, Savar, Dhaka-1213.

Drying and pulverization

About 1 kg of Oyster mushroom and 1 kg of Reishi the mushrooms were cut into small pieces with the help of a knife. These small pieces were sun dried for 7-10 days and finally kept in an electric oven for 72 hours at 40° C. After complete drying, the dried pieces were then pulverized into a coarse powder with the help of a grinding machine (FFC-15, China) and were stored in an airtight container for further use.

Extract preparation

In cold extraction the coarse powder was submerged in ethyl acetate, the common solvents for extracting most of the constituents present in the herbal materials. Flat bottom 2.5 litter reagent bottle were used for this purpose which were kept at room temperature and allowed to stand for 10 days with occasional shaking and stirring. When the solvent become concentrated, the liquid alcohol content was filtered through cotton and then through filter paper (Whatman filter paper no. 1). Then the solvents were allowed to evaporate using rotary evaporator at temperature below 60°C. Thus the highly concentrated and crystalline crude extracts were obtained.

Test Organisms

The bacterial and fungal strains used for the experiment were collected as pure cultures from the Microbiology lab, Dept of pharmacy, University of Rajshahi. Both Gram positive and Gram-negative organisms were taken for the test and they are listed in the Table -1.

Table 1: List of test Bacteria and fungi.

Gram positive Bacteria	Gram negative Bacteria	Fungi
Bacillus cereus	*Escherichia coli*	*Candida albicans*
Bacillus megaterium	*Pseudomonas aeruginosa*	*Aspergillus niger*
Bacillus subtilis	*Shigella sonnei*	*Aspergillus ochreus*
Staphylococcus pyrogens	*Shigella flexneri*	
Streptococcus agalactiae	*Shigella dysenteriae*	

Preparation of medium

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25^0 C) was adjusted at 7.2-7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs. pressure at 121^0 C for 20 minutes. The slants were used for making fresh culture of bacteria and fungi that were in turn used for sensitivity study.

Sterilization procedures

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Air Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121^0C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized [18].

Preparation of subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37^0C for their optimum growth. These fresh cultures were used for the sensitivity test.

Preparation of the test plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial and fungal suspension was immediately transferred to the sterilized Petridishes. The Petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

Preparation of discs

Three types of discs were used for antimicrobial screening.

Standard discs

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Kanamycin (30µg/disc) and Nystatin (30µg/disc) standard disc were used as the reference.

Blank discs

These were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

Preparation of sample discs with test samples

Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

Preparation of sample discs with test samples of Oyster and Reishi extract

Measured amount (400 (µg/disc) of each test sample was dissolved in specific volume of solvent (Ethyl acetate) to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

Application of the test samples

Standard Kanamycin (30 µg/disc) and Nystatin (30 µg/disc) discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

Diffusion and incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow

sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^{0}C for 24 hours.

Determination of antimicrobial activity by measuring the zone of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

RESULTS AND DISCUSSION

In vitro* antimicrobial screening of *Pleurotus ostreatus* and *Ganoderma lucidum

Ethyl acetate crude extract of oyster exhibited moderate activity against the growth of all most all the experimental organisms (Table-2). The reishi ethyl acetate extract exhibited no inhibitory activity against most of the microorganisms (both of the extract concentration of 400 (µg/disc). Ethyl acetate used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper was not active themselves.

Table 2: Antimicrobial activity of test samples extracts.

Test microorganisms	Diameter of zone of inhibition (mm)		
	Oyster extract	Reishi extract	Kanamycin
Gram positive bacteria			
Bacillus cereus	8	2	12.5
Bacillus megaterium	9	3	13
Bacillus subtilis	7.5	1.5	13
Staphylococcus pyrogens	8.5	2	12.5
Streptococcus agalactiae	9	2.5	13
Gram negative bacteria			
Escherichia coli	8	3	14
Pseudomonas aeruginosa	9	4.5	13
Shigellae flexnerii	8	4	12.5
Shigellae dysenteriae	9	5	12
Shigella boydii	8.5	3	14.5
Fungi			**Nystatin**
Candida albicans	-	-	13
Aspergillus niger	-	-	12.5
Aspergillus ochreus	-	-	13

CONCLUSION

The awareness is that the species of *Pleurotus* (i.e., *P. ostreatus*) and *Ganoderma* (i.e., *G. lucidum*) analyzed inhibited the tested bacterial microorganisms in varying degrees. Conversely, both mushrooms didn't show any antifungal activity on supplied test fungi organisms. However, antimicrobial effect of these mushrooms may heavily depend on the nature of solvent in which they were dissolving to make test samples. As a result, further study is necessary by using different solvent system to make the extract for screening these antimicrobial effects *in vitro*.

ACKNOWLEDGEMENT

The present work was supported by the National mushroom development and extension centre, Savar, Dhaka-1213, Bangladesh for giving the selected desired mushrooms in this research work. The authors would like to extend their gratitude to the department of Pharmacy, University of Rajshahi, Bangladesh for providing necessary laboratory facilities to complete of this research successfully.

REFERENCES

1. Sharif MDM, Banik GR. Status and Utilization of Medicinal Plants in Rangamati of Bangladesh. Res. J. Agric. Biol. Sci. 2006; 2(6): 268-273.
2. Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afri. J. Biotechnol. 2007; 6 (14): 90- 1696, (http://www.academicjournals.org/AJB).
3. Manzi, p., Gambelli, L., Marconi, S., Vivanti, V., & Pizzoferrato, L. Nutrients in edible mushrooms: an interspecies comparative study. Food Chemistry. 1999; 65(4), 477–482.
4. Sadler, M. and M. Saltmarsh, Functional foods: The consumer, the products and the evidence. Royal Soc. Chem., Cambridge. 1998.
5. Akyuz, M., A.N. Onganer, P. Erecevit and S. Kirbag. Antimicrobial activity of some ediblemushrooms in the eastern and southeast Anatolia region of Turkey. GU J. Sci., 2010; 23(2): 125-130.
6. Wolff, E.R.S.E., Wisbeck, M.L.L. Silveira, R.M.M. Gern, M.S.L. Pinho and S.A. Furlan. Antimicrobial and antineoplasic activity of *Pleurotusostreatus*. Appl. Biochem. Biotechnol. 2008; 151: 402-412.
7. Kotra, L.P. and S. Mobashery. $-lactam antibiotics, $-lactamases and bacterial resistance. Bull. Inst. Pasteur. 1998; 96: 139-150.
8. Morschhäuser, J., G. Köhler, W. Ziebuhr, G. Blum- Oehler, U. Dobrindt and J. Hacker. Evolution ofmicrobial pathogens. Phil. Trans. R. Soc. Lond. B. 2000; 355: 695-704.
9. Sandven, P. Epidemiology of canidemia. Rev. Iberoam Micol. 2000; 17: 73-81.

10. Thomson, K.S. and E.S. Moland. The new blactamases of Gram-negative bacteria at the dawn of the new millennium. Microbes and Infection. 2000; 2: 1225-1235.
11. Iwalokun, B.A., U.A. Usen, A.A. Otunba and D.K. Olukoya. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. Afri. J. Biotechnol.2007; 6: 1732-1739.
12. Quereshi S, Pandey AK, Sandhu SS. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. J Sci Res. 2010; 3: 9-13.
13. Sheena N, Ajith TA, Mathew AT, Janardhanan KK. Antibacterial activity of three macrofungi, *Ganoderma lucidum, Navesporus floccosa* and *Phellinus rimosus* occurring in South India. Pharm Biol. 2003; 41: 564–567.
14. Gao Y, Tang W, Gao H, Chan E, Lan J, Li X, Zhou S. Antimicrobial activity of the medicinal mushroom Ganoderma. Food Rev Int. 2005; 21: 211-229.
15. Wasser, S.P. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides (minireview). Appl. Microbiol. Biotechnol.2002; 60: 258-274.
16. Rajewska, J. and B. Balasinska. Biologically active compounds of edible mushrooms and their beneficial impact on health. Postepy. Hig. Med. Dosw.2004; 58: 352-357.
17. Lindequist, U., T.H.J. Niedermeyer and W.D. Julich. The pharmacological potential of mushrooms: Evidence based complement. Altern. Med.2005; 2(3): 285-299.
18. Barry, A.L., Principle & practice of Microbiology, Lea & Fabager, Philadelphia. 1976.

HOW TO CITE THIS ARTICLE
Roy DN, Azad AK, Sultana F, Anisuzzaman ASM. *In-vitro* antimicrobial activity of ethyl acetate extract of two common edible mushrooms. The Journal of Phytopharmacology 2016;5(2):79-82.