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Comparative study of antibacterial activity of wood-decay fungi and antibiotics

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Abstract

The antibacterial effects of three mushrooms extract *Ganoderma lucidum, Auricularia auricula, Pleurotus florida* were studied against *Staphylococcus aureus* and *Escherichia coli*. *A. auricula* showed significant antibacterial activity against *S. aureus*. *P. florida* showed some antibacterial activity while *G. lucidum* showed no antibacterial activity. None of the extracts showed any activity against *E. coli*.

Introduction

Whole world is frantically in search of new antibiotics because of an alarmingly increase in bacterial resistance to existing antibiotics due to their inappropriate and indiscriminate use. In search for new antibiotics, herbs and plants are being used.

Mushroom (wood-decay fungi) is considered to have antibacterial activity. Studies have been carried out using different mushroom extracts and different types of microorganisms (Fagade and Oyelda, 2009; Yoon et al., 1994; Quereshi et al., 2010; Gbolagade et al., 2007; Gezer et al., 2006; Sheena et al., 2003; Ishikawa and Kasuya, 2001). The response of microorganisms to mushroom extracts might vary depending upon the nature of environment in which it has been grown (Iwalokum et al., 2007). In this study, mushroom commonly grown in the natural environment of Bangladesh were taken to see their response to microorganisms. Two common microorganisms responsible for infection in everyday clinical practice, *Staphylococcus aureus* and *Escherichia coli* have been taken for the purpose. Most of the studies carried out, so far, showed inhibitory effect of mushroom on different microorganisms. In this study not only the inhibitory effect but also sensitivity pattern of mushroom extract on two microorganisms *S. aureus* and *E. coli* was studied and compared with commonly used antibiotics cloxacillin, cephradine, azithromycin and ciprofloxacin.

Materials and Methods

Two test organisms *S. aureus* (ATCC 22923) and *E. coli* (ATCC 25922) were collected from department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University. Ethanolic extracts of three wood decay fungi *Ganoderma lucidum, Auricularia auricula, Pleurotus florida* were prepared. Mushrooms were collected from National Mushroom Development and Extension Center, Savar, Dhaka, Bangladesh.

Preparation of wood decay extract: Mushrooms were cut, sun dried, grounded into powder and dissolved in

absolute ethanol for 72 hours and stirred every 12 hours. It was then filtered through Whatman filter paper No. 1. The filtrate was concentrated at 40°C using a rotator evaporator (Fagade and Oyelade, 2009). The paste that was formed was freeze dried. Strict sterility was maintained through out the whole procedure. Six different concentration of each wood decay fungi were prepared by taking 75, 125, 250, 500, 750, 1,000 mg in 1 mL of sterile distilled water (for each concentration).

Determination of Minimum Inhibitory Concentration (MIC): Preserved microorganism *S. aureus* was subcultured in blood agar media and *E. coli* was subcultured in MacConcky's agar. Microorganisms were taken from both of these subcultures and inoculated in Muller Hinton agar plates. Different concentrations of wood decay fungi 75, 125, 250, 500, 750, 1,000 mg/mL were taken and agar well diffusion method was applied (Aziz et al., 2007; Gazer et al., 2006; Gbolagade et al., 2007). The plates were left in the room temperature for 1 hour before incubation to allow effusion of the extracts into agar well. Test antibiotics in the strength of cloxacillin 1 µg/disc, azithromycin 15 µg/disc, cephradine 25 µg/disc, ciprofloxacin 5 µg/disc were placed on their particular mark point just before incubation. The discs were incubated at 37°C for 24 hours.

At the end of this period MIC was measured with the help of scale on the under surface petri dish without opening the lid. Zone of inhibition was measured in two directions at right angle to each other from the centre of the disc and average of the two reading was taken.

Interpretation of sensitivity: Zone of Inhibition produced by each antibacterial agents was considered into two categories namely sensitivity (S) and resistant (R) with the help of CLSI (NCCLS) 2010 and as per manufacturers (HIMEDIA).

Results

The zone of inhibition of *S. aureus* against azithromycin, cephradine, ciprofloxacin ranged from 24.0 ± 3.1 to 25.2 ± 1.5 mm respectively and cloxacillin showed minimum zone of inhibition 10.8 ± 0.8 mm (Table I).

In 1000 mg/mL concentration *A. auricula* showed significant zone of inhibition 14.2 ± 1.7 mm, where as *P. florida* exhibited zone of inhibition 12.7 ± 3.3 mm and *G. lucidum* 10.5 ± 0.6 mm in same concentration. *A. auricular*'s zone of inhibition was >13 mm and *P. florida* and *G. lucidum*'s zone of inhibition was <13 mm ($p < 0.05$) compared to cloxacillin, which exhibited minimum zone of inhibition on *S. aureus* and standard zone of inhibition was taken >13 mm. When sensitivity pattern of these extracts were compared with cloxacillin, azithromycin, cephradine and ciprofloxacin, *A. auricula* was significantly sensitive to *S. aureus* and *P. florida* and *G. lucidum* were not sensitive. None of the three extracts showed any inhibition and sensitivity to *E. coli* in highest concentration (Table II).

Table I

Zone of inhibition of wood-decay fungi (mushroom) extracts and drugs discs against two bacteria *Staphylococcus aureus* and *Escherichia coli*

Organisms	Mushroom extracts (mg/mL)	Zone of inhibition (mm)			Drug's disc potency (µg/discs)		Control (Distilled water 60 µL/well)
		Ganoderma lucidum	*Auricularia auricula*	*Pleurotus florida*	Antibiotics	Zone of inhibition	
Staphylococcus aureus (ATCC 22923)	75	0	0	0	Cloxacillin	10.8 ± 0.8	0
	125	0	0	0			
	250	0	0	0	Azithromycin	24.0 ± 3.1	
	500	7.7 ± 1.9	10.8 ± 2.6	8.8 ± 3.5			
	750	9.7 ± 1.4	12.3 ± 3.4	10.5 ± 3.5	Cephradine	24.7 ± 2.2	
	1000	10.5 ± 0.6	14.2 ± 1.7	12.7 ± 3.3	Ciprofloxacin	25.2 ± 1.5	
Escherichia coli (ATCC 25922)	75	0	0	0	Cloxacillin	0	0
	125	0	0	0	Azithromycin	26.7 ± 4.1	
	250	0	0	0			
	500	0	0	0	Cephradine	17.0 ± 2.8	
	750	0	0	0	Ciprofloxacin	40.8 ± 4.1	
	1000	0	0	0			

Doses of cloxacillin (1 µg/discs), azithromycin (15 µg/disc), cephradine (25 µg/discs), and ciprofloxacin (5 µg/discs); Zone of inhibition >13 was taken as significant; Results are shown as mean ± SD

Table II

Sensitivity and resistance pattern of wood-decay fungi (mushroom) extracts and drugs discs against two bacteria *Staphylococcus aureus* and *Escherichia coli*

Organisms	Mushroom extracts (mg/mL)	Mushroom's sensitivity and resistance pattern			Antibiotics sensitivity and resistance pattern		
		Ganoderma lucidum	*Auricularia auricula*	*Pleurotus florida*	Antibiotics used	Sensitivity	Resistance
Staphylococcus aureus (ATCC 22923)	75	(R)	(R)	(R)	Cloxacillin		(R)
	125	(R)	(R)	(R)			
	250	(R)	(R)	(R)	Azithromycin	S (++)	
	500	(R)	(R)	(R)			
	750	(R)	(R)	(R)	Cephradine	S (++)	
	1000	(R)	S (+)	(R)	Ciprofloxacin	S (+++)	
Escherichia coli (ATCC 25922)	75	(R)	(R)	(R)	Cloxacillin		(R)
	125	(R)	(R)	(R)	Azithromycin	S (+++)	
	250	(R)	(R)	(R)			
	500	(R)	(R)	(R)	Cephradine	S (+)	
	750	(R)	(R)	(R)	Ciprofloxacin	S (++++)	
	1000	(R)	(R)	(R)			

Doses of cloxacillin (1 µg/discs), azithromycin (15 µg/disc), cephradine (25 µg/discs), and ciprofloxacin (5 µg/discs); S = Sensitivity; R = Resistance; + = Mildly sensitive; ++ = Moderately sensitive; +++ = Significantly sensitive; ++++ = Highly sensitive; Results are shown as mean ± SD

Discussion

Of the three wood-decay fungi *G. lucidum* showed zone of inhibition similar to that of Cloxacillin, where as *A. auricula* and *P. florida* showed higher zone of inhibition. Significant zone of inhibition was exhibited only by *A. auricula*.

When sensitivity pattern of different wood-decay fungi extracts was compared only *A. auricula* showed significant sensitivity to *S. aureus* (>13 mm). The zone of inhibition and sensitivity of *P. florida* was very near to the standard taken, which is suggestive that higher dose of *P. florida* might show sensitivity to *S. aureus*. *G. lucidum* showed resistance. None of the extracts showed inhibition and sensitivity to test organism *E. coli*.

In a more or less similar study (Fagade and Oyelade, 2009) inhibitory effect of *G. lucidum, A. auricula* and *P. florida* was observed against *S. aureus* and *E. coli*, where *A. auricula* and *P. florida* showed antibacterial activity against both the organisms. In this study only *A. auricula* showed significant and *P. florida* showed moderate inhibitory effect and sensitivity to *S. aureus*. None of the three extracts exhibited any inhibitory or sensitivity to *E. coli*.

This difference in response of mushroom extracts to test organisms might be due to a number of factors, as studies suggest that the antimicrobial activities of all mushroom extracts are changeable (Iwalokun et al., 2007), depending upon the nature of environment and media in which it is grown. It also depends upon the genetic structure of mushroom species, physical and biochemical constituent's differences of mushroom extracts solvents and test organisms. The sensitivity pattern of microorganisms also changes to chemotherapeutic agents depending on their strains, and susceptibility or resistance to antibiotic (Gao et al., 2005).

Studies using different test organisms and different mushroom extracts also showed that mushroom posses antibacterial activity to varying degrees (Gazer et al., 2006; Upadhyay et al., 2010; Kim et al., 2001). Antibacterial activity of mushroom extracts have been attributed to presence of biologically active compounds. Studies suggest that these biologically active compounds enhance immunity (Ramesh and Pattar, 2010) and they also posses antitumor properties. The antibacterial and antitumor properties of mushroom have been attributed to presence of polysaccharides, terpens and lectins. Studies suggested that polysaccharides from mushrooms do not act directly but modify the different immune responses in the host mainly by increasing macrophage activity that destroy pathogens such as bacteria, virus etc (Wasser and Weis, 1999). In addition to their immunomodulating effect they have significant cardioprotective, antiparasitic, hepatoprotective and antidiabetic effect (Wasser and Weis, 1999).

Mushroom taken as food has the advantage of being devoid of dose related adverse effect seen with antibiotics. Resistance to antibiotics due to its frequent and inappropriate use pose a threat to treatment of bacterial infections mostly in under developed countries and in many developed countries of the

world as well. Therefore mushroom with antibacterial properties have received considerable attention in recent years. Search for new antimicrobial agents has gained considerable importance, and mushrooms for their antibacterial activity may be considered for their easy availability and cheapness. Some times antibiotic combinations are being used to overcome the problems of drug resistance (Lewin et al., 1991; Kim et al., 2001), but use of two or more antibiotics carries more chance of antibiotics related adverse effect and use of mushroom along with antibiotics might help lessen the adverse effects (Kim et al., 2001).

Conclusion

Mushroom extracts can be used to combat pathogenic microorganisms along with available antibiotics.

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