Evaluation Of Susceptibility Of Methanol Extract Of Pleurotus Highking, An Edible Mushroom Cultivated In Bangladesh

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ABSTRACT: The methanol extract of an edible mushroom, Pleurotus highking with its whole parts, cap and stipe individually were subjected for the evaluation of antimicrobial activities against a series of pathogenic microorganisms. Three Gram positive (Bacillus cereus, Streptococcus agalactiae and Agrobacterium), three Gram negative (Pseudomonas aeruginosa, Escherichia coli and Shigella dysenteriae) and three pathogenic fungus (Aspergillus niger, Saccharromyces cerevaceae and Candida albicans) were used as test pathogen. Disc diffusion assay method was employed for our experimental purpose. All the tested Gram positive strains were sensitive to 200µg of extracts/disc while there was no sensitive to Gram negative strain, Escherichia coli and Shigella dysenteriae. A tested Gram negative strain, Pseudomonas aeruginosa also sensitive to the extracts at a concentration of 200µg/disc. Aspergillus niger and Candida albicans were susceptible to methanol extract of Pleurotus highking while Saccharromyces cerevaceae was resistant to it. However, the susceptibility of the extracts from different parts was varied. The extract from cap showed prominent antimicrobial activity as compared to whole mushroom and stipe.

Index Terms: Antibacterial, Antifungal, Kanamycin, Mushroom, Pleurotus highking, Resistance, Susceptibility

1. INTRODUCTION:

From ancient times mushrooms are recognized as food items. Mushrooms contain a huge diversity of biomolecules with nutritional [2] and/or medicinal properties [3, 7 and 8]. Due to these properties, they have been recognized as functional foods, and as a source for the development of medicines and nutraceuticals. The development of antibiotics has been one of the most important scientific achievements of the last seventy years. Despite the huge diversity of antibacterial compounds, bacterial resistance to first-choice antibiotics has been drastically increasing. Moreover, the association between multiresistant microorganisms and nosocomial infections highlight the problem, and the urgent need for solutions. Natural resources have been exploited in the last years and among them, mushrooms could be an alternative source of new antimicrobials. Data available from the literature indicate a higher antimicrobial activity of mushroom extracts against gram-positive bacteria.

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Among all the mushrooms, Lentinus edodes is the most studied species and seems to have a broad antimicrobial action against both gram-postive and gram-negative bacteria. Plectasin peptide, obtained from Pseudoplectania nigrella, is the isolated compound with the highest antimicrobial activity against gram-positive bacteria, while 2-aminoquinoline, isolated from Leucopaxillus albissimus, presents the highest antimicrobial activity against gram-negative bacteria. In particular, mushrooms could be a source of natural antibiotics [11], which can be Low molecular weight (LMW) and High Molecular Weight (HMW) [1] respectively, compounds. LMW compounds are mainly secondary metabolites such as sesquiterpenes and other terpenes, steroids, anthraquinone and benzoic acid derivatives, and quinolines, but also primary metabolites such as oxalic acid and HMW compounds mainly include peptides and proteins [6]. As a part of our study to search of antibacterial compounds from the natural sources, the current investigation was undertaken to screen antibacterial and antifungal activity of crude methanolic extracts of Pleurotus highking, an edible mushrooms commercially cultivated in Bangladesh.

2. MATERIALS AND METHODS

2.1 Sample Collection:

The Pleurotus highking mushroom was collected from National Mushroom Development and Extension Centre, Savar, Dhaka 1340, Bangladesh. The sample was dried at room temperature for 12 days, then placed in locked bags and stored at 25^{o} C.

2.2 Extraction of crude extract:

The dried mushroom was crushed in the grinding machine to powder and these powdered materials (50gm) were mixed with 250ml of methnol in a beaker and placed on a rotary shaker for 24 h. The methanol content was filtered using Whatman filter paper (No 4) and the solvent was evaporated for 15 min at 37°C using a Rotary evaporator and crude extract was obtained. To get the sample solution

0.5gm of crude extract was mixed with 25ml of methnol. Each 10µL solution (200µg of extract) was impregnated into a sterile filter paper disc having a diameter of 6mm.

2.3 Collection of Pathogenic Bacteria:

A total of six pure strains of pathogenic bacteria (three Gram positive bacterial strains; Bacillus cereus, Streptococcus agalactiae and Agrobacterium and three Gram negative bacterial strains; Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae) were collected from the "International Centre for Diarrheal Disease Research' Bangladesh (ICDDR'B). Three fungal strains; Aspergillus niger, Saccharromyces cerevaceae and Candida albicans were also collected from the ICDDR'B.

2.4 Antimicrobial Activity

The antimicrobial activity of the extracts was carried out by the disc diffusion assay method [4]. The bacterial cultures were maintained in nutrient broth media. 100µL of each culture was uniformly distributed on Yeast Extract Glucose Agar plates. Sterile filter paper discs impregnated with extracts were placed on the surface Yeast Extract Glucose Agar plates using sterile forceps. Kanamycin (30 µg/disc) was used as positive control. The plates were kept at 4°C for 4 hour for the diffusion of the extracts. The inoculated plates were incubated at 37^{o}C for 24 hour. After incubation period, the zones of inhibition were measured using scale [4].

2.5 Antifungal Activity

Antifungal activity was also performed by disc diffusion assay method [4]. The fungal cultures were maintained in Sabouraud's dextrose broth. 100µL of each culture was uniformly distributed on Sabouraud's dextrose agar plates. Sterile filter paper discs (6mm) impregnated with the test sample and dried by hairdryer. The discs were placed on the surface on Sabouraud's dextrose agar plates. Methanol itself was introduced into the disc and was used to nullify the effect of solvent. Griseofulvin (30 µg/disc) was used as positive control. The plates were incubated at 4°C for 4 hour for diffusion. The inoculated plates were incubated at 25^{o}C for 48 hour and zone of inhibition was observed. Diameters of the zone of inhibition obtained from the different extracts were compared than that of the standard.

3. RESULTS

In order to determine the comparative efficacy of the methanolic extracts of entire mushroom, cap and stipe was tested against the test pathogenic bacterial strains. Results are shown in the Table 1. All the test extracts exhibited predominant antibacterial property to Gram positive bacteria as compared to Gram negative bacteria. Among the extracts, cap extracts were more effectively inhibited the bacterial growth. The tested pathogenic Gram positive bacteria showed almost similarly sensitive to the extracts of P. highking. While among the Gram negative bacteria, Pseudomonas aeruginosa were moderately sensitive to the entire mushroom, the cap and the stipe extracts. Escherichia coli and Shigella dysenteriae were resistant to the entire mushroom and the cap extracts, and poorly sensitive to the cap extracts.

Table 1 Antibacterial activity of entire, stipe and cap extracts against a series of test bacteria.

Test organisms	Diameter of zone of inhibition (mm)			
	Entire extracts	Stipe extracts	Cap extracts	Positive control
Gram-positive bacteria:				
Bacillus cereus	14 ± 0.41	14 ± 0.36	17 ± 0.36	25 ± 0.26
Streptococcus agalactiae	12 ± 0.35	13 ± 0.32	18 ± 0.36	21 ± 0.30
Agrobacterium	11 ± 0.22	12 ± 0.26	16 ± 0.42	22 ± 0.28
Gram-negative bacteria:				
Pseudomonas aeruginosa	11 ± 0.25	11 ± 0.30	14 ± 0.25	20 ± 0.31
Escherichia coli	-	-	09 ± 0.35	18 ± 0.41
Shigella dysenteriae	-	-	08 ± 0.30	20 ± 0.32

The values expressed as mean ± SEM of 3 -4 experiments. " – " indicates no inhibition .

The obtained results indicate a difference in antimicrobial activity among the extracts. The agar diffusion bioassay showed that cap of the mushroom extract have the highest activity against all Gram-positive bacteria and they also showed good activity against Gram-negative bacteria. The reason for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da [9]. The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier [10]. The results are of interest since they have been obtained with crude extracts and are not a pure product and it could be

considered to have a good potency level after isolating the antibacterial principles upon chromatographic analysis. To determine the antifungal activity of the extracts of entire mushrom, stipe and cap was selected against the pathogenic fungus, Aspergillus niger, Saccharromyces cerevaceae and Candida albicans by disc diffusion assay method. The results are shown in the Table 2. Candida albicans and Aspergillus niger were sensitive to the extracts of the entire mushroom and stipe. Interestingly, the mentioned two extracts were failed to show antifungal activity against Saccharromyces cerevaceae. Our result also exhibited moderate activity against Candida albicans and Aspergillus niger as compared to the standard and negligible effect against Saccharromyces cerevaceae. Variations in the antifungal effectiveness of different extract against different fungi were mostly likely due to difference in nature of inhibitors materials they contained. The findings of this study suggest that the mushroom P. highking is important source of compounds that are effective against some pathogenic fungi.

Table 2 Antifungal activities of entire, stipe and cap extracts against a series of pathogenic fungi

Test organisms	Diameter of zone of inhibition (mm)			
	Entire extracts	Stipe extracts	Cap extracts	Positive control
Aspergillus niger	08 ± 0.52	10 ± 0.39	12 ± 0.37	18± 0.36
Saccharromyces cerevaceae	-	-	07 ± 0.26	16± 0.42
Candida albicans	09 ± 0.57	11 ± 0.15	13 ± 0.62	17± 0.57

The values expressed as mean ± SEM of 3 -4 experiments. " – " indicates no inhibition .

4. CONCLUSION

Among the three extracts, the cap extract exhibited maximum antibacterial activity against Bacillus cereus, Streptococcus agalactiae, Agrobacterium and Pseudomonas aeruginosa. The remaining other two extracts (entire and stipe) exhibited moderate antibacterial activity against these pathogenic strains, while bacterial strains Shigella dysenteriae and Escherichia coli were seems to be resistant to all of three extracts. The order of anitifungal activity against Aspergillus niger and Candida albicans were cap extract> Stipe extracts> entire extracts. While the Saccharromyces cerevaceae was resistant to the tested extracts except cap extract. Demirhan et al., 2007 [5] reported antibacterial activity of P. ostreatus. In their study acetone extract of P. ostreatus did not present an antimicrobial effect against E. coli, S. aureus and P. aeruginosa. In our study the extracts are susceptible to P. aeruginosa, mean while resistant to E. coli. However, to the best our knowledge, yet no report were observed regarding the antibacterial activity of Pleurotus high king. It was concluded that the antimicrobial activity is direct correlated with the content of different portions. The extracts of different portion of this mushroom are recommended to use and the mushroom cap proved to be better than the entire mushroom and stipe. A little is known on the in vitro antimicrobial activities of Bangladeshi edible mushrooms (entire, cap and stipe) specially the Pleurotus high king. Research is going on to isolation of the bioactive metabolites from edible mushrooms. After elucidation of their mechanism of action, these mushroom metabolites or other related compounds could be used to develop nutraceuticals or drugs effective against pathogenic microorganisms resistant to conventional treatments. Mushroom extracts could be an alternative as antimicrobials against pathogenic micro-organisms resistant to conventional treatments

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