Antimicrobial Activity of Selected Malaysian Mushrooms Against *Staphylococci a*nd *Streptococci*

Al Dhalli S.[1], Pavithira P. R.[1], Jiyauddin K.[1], Kaleemullah M.[1],Mohd Fadli A.[1]Eddy Y. [1]
[1]*School of Pharmacy, Management & Science University, Selangor Darul Ehsan, Malaysia*

Keywords: *Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus bisporus*, *Staphylococci* and *Streptococci.*

Abstract : One of the biggest problems in the developing world is the proliferation of treatable bacterial infections. The use of mushrooms as nutritional food as well as medicine is gaining popularity. The aim of this research is to evaluate the antimicrobial activity in *Lentinula edodes*, *Pleurotus ostreatus* and *Agaricus bisporus* against *Staphylococcus* and *Streptococcus* microorganism. The mushroom was dried and grounded into powder and extracted by cold maceration. The solvent was extracted through evaporation and then the extract was tested for its antimicrobial activity against *Staphyloccoci* and *Streptoccoci* microorganism by disc Diffusion Method. The chloroform extract of Shiitake mushroom exhibited maximum zone of inhibition against *Streptococci* species was 14 mm. Moderate activity of acetone extracts against *Streptococci* species was observed as 12 mm. The chloroform extracts showed broad spectrum of antibacterial activity against the tested bacterial pathogens than other solvents. It was found that the shiitake mushroom has antibacterial activity and it gives reliable indication of the concentration of drug required to inhibit the growth of microorganism compared with to the other two types of mushrooms. Shiitake mushrooms cultivated in Malaysia are potential sources of bioactive compound against streptococcus species and should be investigated for natural antibiotics.

1 INTRODUCTION

Microbes are tiny organisms which cannot be seen without a microscope, yet they are abundant on Earth. They live everywhere such as in air, soil, rock, and water (NIAID,2010). Some microbes need oxygen to live, but others do not. These microscopic organisms are found in plants and animals as well as in the human body. Some microbes cause disease in humans, plants, and animals. Others are essential for a healthy life, and we could not exist without them. Indeed, the relationship between microbes and humans is delicate and complex. Microbes make up more than 60 % of the Earth's living matter and scientists estimate that 2-3 billion species share the planet with us.

Most microbes belong to one of four major groups: bacteria, viruses, fungi, or protozoa. Since the 19th century, we have known microbes cause infectious diseases. Near the end of the 20th century, researchers began to learn that microbes also contribute to many chronic diseases and conditions. Mounting scientific evidence strongly links microbes to some forms of cancer, coronary artery disease, diabetes, multiple sclerosis, and chronic lung diseases (NIAID,2010).

One of the biggest problems in the developing world is the proliferation of treatable bacterial infections which run unchecked through populations due to lack of access to medications and medical treatment. Therefore, suitable action must be taken at the right time to combat the problem. For example, developing new drugs either synthetic or natural rather than using of antibiotic only for treating infectious disease. The use of mushrooms as nutritional food as well as medicine is gaining popularity in recent times. The nutritive and medicinal properties of many mushrooms have been documented. Few examples of mushrooms which are known for its antimicrobial activities are *Letinula edodes* , *Pleurotus ostreatus* and *Agaricus bisporus.*

Lentinula edodes (shiitake) an edible mushroom indigenous to East Asia, is cultivated worldwide for its purported health benefits. Lentinan ([1,3] beta-D-glucan), a polysaccharide isolated from shiitake, is thought to be responsible for many of the mushroom's beneficial effects.(Barrie, 2011).Shitake

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mushrooms have both medicinal and culinary properties which encourages the plantation of it throughout the world. *Lentinula edodes*posses antibacterial effects against bacteria such as *Streptococcus spp*., *Actinomyces spp*., *Lactobacillus spp* and also against cancer. Apart from it *L.edodes* also has antitumour, antiviral, hypocholesterolemic and hypoglycemic properties which plays an important role in the consumption of these mushrooms.

Apart from it, *Pleurotus ostreatus* (oyster) are also another common type of mushroom which has been extensively used in traditional Chinese medicine from as early as 3,000 years ago. These mushrooms commonly used due to huge advantages which include its nutritional value and other medicinal benefits which presents in it. In detailed, oyster mushrooms posses an antioxidant property as well as antibacterial activities which against various species of Gram positive and Gram negative bacteria. Besides, *Pleurotus ostreatus* present with an antitumor activities which may be effective for improving antioxidant capacity and preventing tumor.

In addition, *Agaricus bisporus* (button mushrooms) are also included in the category of medicinal mushrooms due to some evidence such as the results of a study, published in the July 2010 issue of "Nutrition Journal," show that button mushrooms reduce inflammation in arterial cells and prevent white blood cells from sticking to arterial walls.(Traci, 2011) The researchers conclude that consuming button mushrooms may be a means to prevent heart disease and also released a report stating that button mushrooms have been found effective at treating breast, colon and prostate cancers.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Mushrooms

Lentinula edodes, *Pleurotus ostreatus* and *Agaricus bisporus* **Bacteria strain** – *Staphylococci and Streptococci* **Antibiotic** Amoxycillin

2.1.2 Other Materials

70% Ethanol , distilled water , Acetone , Chloroform , Whatsman filter paper No 60 , beaker , blender , incubator , Mueller Hinton Agar , Sterile Petri dish ,test tubes , nutrient broth , aluminium foil , sterile cotton swab , Bunsen burner , weighing balance , spatula ,filter funnel , conical flask , measuring cylinder , micropipette and ruler.

2.2 Methods

2.2.1 Collection of Samples

The *Lentinula edodes*, *Pleurotusostreatus* and *Agaricusbisporus* mushrooms was collected from a supermarket.

2.2.2 Preparation of Powder

The collected mushrooms were washed with running tap water to remove adhering materials. Then, the mushrooms were sliced and dried at temperature not exceeding 50 ºC.

These dried materials were pulverized mechanically into coarse powder. The fine powder was separated by passing through sieve No. 60. The coarse powder obtained was used for the extraction process.

2.2.3 Preparation of Extracts

The coarse powdered of mushrooms such as shiitake, oyster and white button mushrooms (250gm) were taken in an aspirator bottle separately and extracted successively by cold maceration technique with solvents like aqueous, chloroform, acetone and ethanol respectively for six days.At the end of each extraction they were filtered through filter paper. Except aqueous extract all other extracts were distilled over water bath to remove 80% of the solvent. The aqueous extract was concentrated by distilling on a mantle. The remaining portion of all the solvents from the extracts was removed under vacuum.

2.2.4 In-vitro Antibacterial Screening for Extracts by Disc Diffusion Method

The antibacterial activity of the extract determined by streaking bacterial cultures with a nutrient agar medium in petri plates. Sterilized filter paper discs (Whatman No 1) soaked in different beakers containing the dissolved extracts of different mushrooms were taken out with sterilized forceps and air-dried and placed on plates with the different organisms such as Staphylococcus and

Streptococcus. The plates were incubated at 37°C for 24 h for bacterial strains. After incubation, the inoculated plates were observed for zones of

inhibition in millimeter diameter using a transparent ruler. The sensitivity or susceptibility of the test bacteria to the standard drug was tested using an inoculated agar plate and Amoxicillin 250mg. The zones of inhibition were measured and compared with those of the plant extract.

Table 1: Antimicrobial effect of different types of mushrooms, in different types of solvents against Staphylococci and Streptococci.

Zone of inhibition / Types of solvents	**Zone Of Inhibition After 24 hours (mm)**					
	Shiitake Mushroom		**Oyster Mushroom**		**White Button Mushroom**	
	Staphylococci	*Streptococci*	*Staphylococci*	*Streptococci*	*Staphylococci*	*Streptococci*
Acetone	0	12	0	0	0	0
Chloroform	0	14	0	0	0	0
Ethanol	0	0	0	0	0	0
Amoxycillin (positive)	17	20	17	20	17	20
Distilled Water (negative)	0	0	0	0	0	0

2.2.5 Determination of Minimal Inhibitory Concentration(MIC)

The determination of MIC extracts will be measured by using a tube dilution method with a slight modification. Briefly, extracts will be subjected to a series of serial dilution. Extracts with different concentration will be added aseptically into different labeled test tube containing sterile Muller Hinton broth. Then,the bacterial suspension and fungal suspension will be inoculated into respective test tubes. The test tubes will be incubated bacteria at 37^0c for 21-24hours and fungus at 28^0c for 24-28hrs.The MIC value will be measured by comparing turbidity of the whole series of test tubes with a negative control and positive controls.MIC value will be stated as the highest concentration that shows no turbidity which indicates no growth of bacteria. Each test will be performed in triplicate. The tube dilution test is the standard method for determining levels of resistance to an antibiotic. Serial dilutions of the antibiotic are made in a liquid medium which is inoculated with a standardized number of streptococcus organisms and incubated for 72 hours time. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). At this dilution the antibiotic is bacteriostatic. First of all, test tubes numbered from 1 to 4. Then,1.0 ml of sterile broth added to all test tubes.2.0 ml of shiitake acetone extraction and 1ml of streptococcus organism suspension added to the first test tube(100 mg/ml).1.0 ml of the contents from the first tube transferred to the second tube. Using a separate pipette, the contents of this tube mixed and transferred 1.0 ml to the third tube. Continue dilutions in this manner to tube number 4, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette. Finally 1.0 ml from tube 4 is removed and discarded. Same process followed for shiitake chloroform extraction and all test tubes incubated at 35ºC for 72 hours. After the determined period , tubes examined for visible signs of bacterial growth. The highest dilution without growth is said to be the minimal inhibitory concentration (MIC).

3 RESULTS

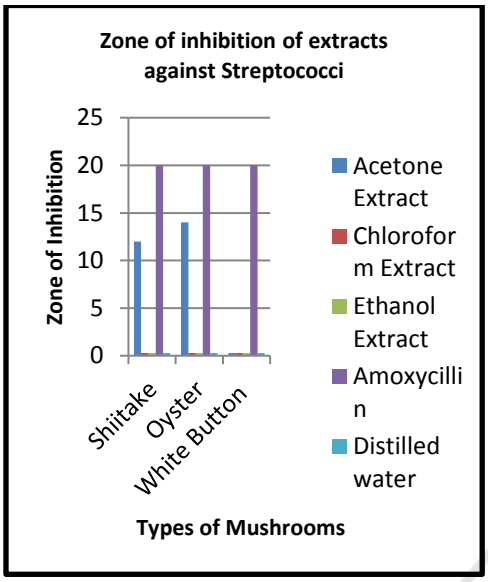

Figure 1 Antimicrobial effect of extracts and controls against Streptococci

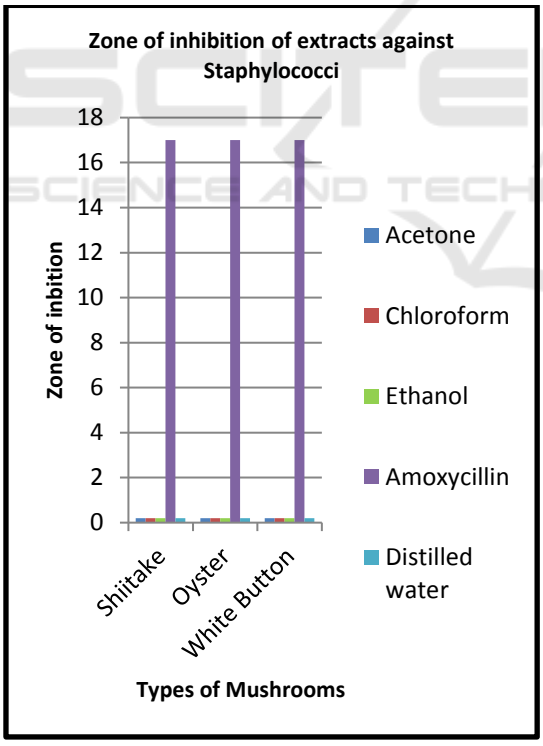

Figure 2 Antimicrobial effect of extracts and controls against Staphylococci

4 DISCUSSION

In the present investigation, the antibacterial properties of chloroform,acetone,alcohol and aqueous extracts of medicinal mushrooms such as shiitake,oyster and white button mushrooms were tested against human pathogenic bacteria. The antibacterial properties of the extracts were also comparatively analysed against standard antibiotics by antibiotic sensitivity test.

The chloroform extract of shiitake mushroom exhibited maximum zone of inhibition against streptococcus species was 14mm. The moderate activity of acetone extracts against streptococcus species was observed as 12mm. The chloroform extracts showed broad spectrum of antibacterial activity against the tested bacterial pathogens than other solvents.

The medicinal mushrooms such as white button and oyster against staphylococcus and streptococcus species resistance against solvent extraction of chloroform, acetone, ethanol and aqueous. Similarly, shiitake against staphylococcus species also resistance against all the solvent extractions.

The antibiotic sensitivity test using standard antibiotic which is amoxycillin were tested against pathogenic bacteria. The antibiotic used was exhibited antibacterial activity. The results confirmed that the solvent extracts such as chloroform and ethanol of shiitake mushroom exhibited a higher antibacterial activity against streptcoccus pathogenic bacteria. The result of antibacterial effect of chloroform, acetone, ethanol and aqueous solvents of white button and oyster mushrooms revealed no activity against pathogenic bacterial strains.

Thus, MIC assay are capable of verifying that the shiitake mushroom has antibacterial activity and that it gives reliable indication of the concentration of drug required to inhibit the growth of microorganism. Acetone and chloroform extract of shiitake mushroom was subjected to get the MIC against strpetococcus species and it was found to be 50mg/ml for acetone extract and 12.5mg/ml for chloroform extract.

5 CONCLUSION

The in- vitro comparative study of antimicrobial activity of selected Malaysian mushrooms against Streptococci and Staphylococci in different solvent

extract concludes that the chloroform extract of shiitake mushroom exhibited maximum zone of inhibition against Streptococcus species was 14mm. The moderate activity of acetone extracts against Streptococcus species was observed as 12mm. The chloroform extracts showed broad spectrum of antibacterial activity against the tested bacterial pathogens than other solvents.

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CONFLICT OF INTEREST

Author declared there is no conflict of interest.

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