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Preliminary Detection of Pharmacological Properties of *Polyporous sanguineus* Isu Strain Using Different Indigenous Liquid Substrates

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Polyporus sanguineus is colourful mushroom belonging to Family Polyporaceae that acts as a synthesizer of some pigments. It has been reported to have antibacterial properties with good therapeutic potential and pharmacological application. This study was carried out to determine the growth performance of its mycelia on three different indigenous liquid substrates and evaluate its antibacterial activities. Naturally occurring fruiting bodies of *P. sanguineus* were collected inside the university and were immediately isolated using the standard laboratory procedure on tissue culture. Five (5) day old mycelia were inoculated and cultured in flask containing three indigenous liquid media which includes mung bean decoction (mbd), coconut water broth (CWB) and potato dextrose broth (PDB). After seven (7) days of incubation, formed mycelial mats of *P. sanguineus* were strained, air dried and pulverized. Pulverized mycelial mats from different liquid substrates were subjected to ethanolic extraction for antimicrobial assay activities. Results showed that mung bean decoction (MBD) produced the thickest mycelial mats compared to potato dextrose broth (PDB) and coconut water broth (CWB). On the other hand, antimicrobial assay revealed that *P. sanguineus* aqueous extracts is a potential antibacterial agent against *E. coli* (36.40 mm) and *S. aureus* (33.36 mm).

Keywords: Indigenous, *Polyporous sanguineus*, liquid substrate Pharmacological

Introduction

Mushrooms are ignored by many, reviled by some and may turn out to be important keys to both human health and planetary health (Staments, 2005). With good therapeutic potential and pharmacological application, Polyporus fungi are listed as food and cosmetics grade microorganisms and emerged in the early 90's as a genus whose biochemistry, biotechnological properties have since been progressively detailed (Lomascolo *et al.*, 2011). *P. sanguineous* is one of the four representative species of the genus *Polyporous*. It occurs in tropical and subtropical regions of Northern and Southern

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hemispheres. This species has been shown as lacase producer with high redox potential (Sullivan *et al*, 1971).

Many antibiotics in clinical use were developed from fungal and actinomycetes metabolites. During the last decades several pathogenic microorganisms developed resistance to the available antibiotics. Infections by multidrug resistant isolates of *Candida* sp., *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus* sp., *Enterococcus* sp. and *Escherichia coli*, among others, became more and more frequent stimulating the search for new antibiotics with novel mechanisms of action (Kotra and Mobashery 1998, Morschhäuser *et al*., 2000, Sandven 2000, Thomson and Moland 2000).

The first investigations on the potential of basidiomycetes as sources of antibiotics were performed by Anchel, Hervey, Wilkins in 1941 (Sandven 2000), when they examined extracts of fruiting bodies and mycelia culture from over 2000 species. They succeed in the isolation and identification of pleuromutilin (Kavanagh *et al*., 1950), a diterpene that is especially useful for the treatment of mycoplasm infections in animals (Brizuela *et al*., 1998) and served for the development of the first commercial antibiotic of basidiomycetes origin.

Moreover, interest in the metabolites produced by basidiomycetes declined as streptomycetes were considered to be a more prolific and easier to manipulate source of antibiotics (Anke, 1989). However, over 6000 metabolites were already identified from these imperfect fungi, making it more and more difficult to isolate novel bioactive metabolites from them. With the development of new fermentation and purification technologies, basidiomycetes are again receiving attention as potential sources of new classes of antibiotics (Anke 1989, Maziero *et al*., 1999, Suay *et al*., 2000). In relation, this study aimed to assess the growth of mycelia on three different liquid substrates and to evaluate the antibacterial potential of *P. sanguineus*.The antimicrobial activity of *P. sanguineus* has been known since 1946, when Bose (1946) isolated poliporin, a compound active against Gram-positive and Gram-negative bacteria and without toxicity to experimental animals. More recently, studies by Smânia *et al*. (1995) showed that this basidiomycetes produces cinnabarine, an orange pigment active against *B. cereus, E. faecalis, E. faecium, E. coli, K. pneumoniae, L. mesenteroides, L. plantarum,P. aeruginosa, Salmonella* sp., S. *typhi, S. aureus* and several *Streptococcus* spp. Cinnabarine was more active against Gram-positive than against Gram-negative bacteria. According to Fidalgo (1965) some Brazilian indigenous people use the basidiomes of *P. sanguineus* to stop haemorrhages).

Materials and Methods

Microorganism and inoculum preparation

P. sanguineus (Isabela strain) was collected from the premises of Isabela State University. Fruiting bodies were subjected to morphological identification using published articles and recorded taxonomic keys. Tissues were isolated following standard microbiological procedures to obtain pure culture. Stock cultures of *P. sanguineus* were deposited to Microbiology Laboratory of Science Building, Department of Biological Sciences at Isabela State University, Echague Campus maintained on potato dextrose agar (PDA). From the stock cultures, mycelial blocks were prepared and incubated for five days. Flasks containing liquid substrates were inoculated with 5-day old mycelium of *P. sanguineus* grown on PDA and incubated at room temperature (28-30°C).

Liquid Media Preparation

Mung bean decoction

Five hundred grams (500g) of mung bean was boiled on 1 Liter of distilled water added with 20 g of sugar. Broth was separated using strainer. Approximately 50 ml of the mixture was dispensed a previously sterilized flask, plugged with cotton, sterilized using autoclave at 121^{o}C, 15 psi for 15 minutes.

Coconut water broth

Fresh mature coconut water was obtained from the public market of Santiago City, Isabela. The coconut water was filtered to remove impurities. Approximately 50-60 ml of filtered coconut water was placed on a sterile flask plugged with cotton, sterilized using autoclave at 121^{o} C, 15 psi for 15 minutes.

Potato dextrose broth

Fresh potatoes weighing 250g were cut into halves, boiled with 1Liter of distilled water until tender. Mixture was reconstituted with distilled water prior addition of 10g of sugar. Prepared mixture was dispensed on a sterile flask plugged with cotton, sterilized using autoclave at 121^{o}C, 15 psi for 15 minutes.

Inoculation

Approximately 10 mm agar disc of *P. sanguineous* were inoculated into flasks containing liquid substrates. After seven days, culture on each flasks were separately strained to get the fresh mycelial mats. The mats were air dried at room temperature (28-30°C) for 3 days. Fresh and dried

mycelia mats were separately weighed (g) using analytical balance prior pulverizing which will be used for aqueous extraction (Sibounnavoung, *et al.*, 2009).

Preparation of Extracts

For aqueous extraction, 10g of air-dried mycelial powder was added to 100 ml distilled water and subjected on slow heat (70^{o} C) for 2 hours. It was then filtered using Whatman filter paper no. 1 to obtain filtrate samples. The samples were placed in a flask and subjected further to hot bath for 1 hour to obtain the final volume aqueous extracts and stored for further use for antibacterial activities.

Disc diffusion method

Previously prepared Petri plates containing sterile Mueller-Hinton Agar were inoculated with a standardized inoculum of the test microorganisms (*E. coli* and *S. aureus*). Approximately 6mm paper discs containing the test compound was inoculated to each plate. Inoculated plates were incubated at room temperature (28-30 °C). Zones of inhibition were measured using digital Vernier calliper every 8 hours for a period of 24 hours.

Results and Discussion

Mycelial mat performance of P. sanguineus on three indigenous liquid broth

P. sanguineus Isabela strain was cultured on a flask using three different indigenous liquid substrate, coconut water broth (CWB), potato dextrose broth (PDB) and mung bean decoction (MBD). As shown on Figure 1, results revealed that fresh weight of *P. sanguineus* on MDB produced the thickest (61.6g) mycelial mat while CWB produced only 10.2 g of fresh mycelial mats. However, PDB produced the least weight of 2.00 g mycelial mat. Sibounnavoung *et al*. (2009) stated that this result is due to the presence of bioactive components of the liquid substrates.

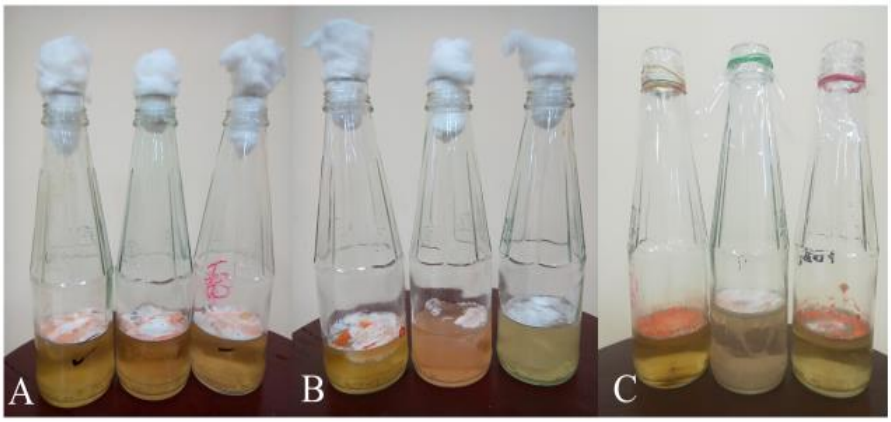

Figure 1. Indigenous Liquid Substrates: (A) Mycelial Mat in Mongo decoction broth; (B) Mycelial Mat in Coconut Water Broth and (C) Mycelial mat in Potato dextrose broth.

Effect of pH

Results also showed that slightly acidic pH has supported the growth of *P. sanguineus* (Table 1). pH is an important factor for the growth of microorganisms such as fungi. However, fungi could grow over a wide range of pH 3 to 11. Both lower and higher levels of pH showed adverse effect on mycelia growth (Kapoor and Sharma, 2014).

Table 1. Mean weight of *P. sanguineus* mycelial mat as affected by different pH levels

pH levels	Weight of mycelial mat (g)		
	MBD	**CWB**	**PDB**
5.5	42.15^{c}	10.20^{a}	1.27^{d}
6.0	61.60^{a}	7.65^{c}	2.00^{a}
6.5	57.85^{b}	8.21^{b}	1.43^{c}
7.0	32.26^{d}	5.43^{d}	1.87^{b}
7.5	23.21^{e}	3.43^{f}	1.12^{e}
8.0	12.15^{f}	4.39^{e}	1.10^{f}

Note: Means with the same superscript in the column are insignificantly different from each other at 5% level of significance.

Based on the results gathered from Table 1, this segment exudes MBD at pH 6.0 (61.60) supported the luxuriant growth of *P. sanguineus* mycelial mat. On the other hand, CWB at pH 5.5 revealed 10.20g as its best weight. PDB exhibited 2.00 g as its best weight at pH 6.0. Among the three indigenous liquid substrates used, it was observed that MBD exhibited the highest mean weight at pH 6.0 as compared to CWB and PDB. The stimulating effect of the three sources can be attributed to their carbon and nitrogen content and mycelia production in *P. sanguineus* influenced by different nutritional requirements as suggested by Sibounnavoung *et al*. (2009).

According to Levi (1968), wood decaying basidiomycetes have evolved extremely efficient mechanism for assimilating the nitrogen available in wood and soil and then recycling the nitrogen by autolysis into new, actively growing portions of mycelium. However, the results of this study contradicts the findings of Hackskaylo (1954) that basidiomycetes with the exception of *Polyporous distortous* utilize nitrate nitrogen very slowly. In this present study *P. sanguineus* grown luxuriantly in a nitrogenous liquid substrate.

Disc diffusion assay

Aqueous extracts of *P. sanguineus* were evaluated for its antibacterial potential against test bacteria *E. coli* and *S. aureus* using agar disc diffusion method. The three test compounds were determined to suppress the growth of both *E. coli* and *S. aureus* and possess inhibitory activities against the test microorganisms.

Table 2. Antibacterial assay of aqueous extracts of *P. sanguineus* against *S. aureus* and *E. coli*.

Treatment	Antimicrobial Assay	
	S. aureus (mm)	***E.coli*** (mm)
Distilled water	6.00^{c}	6.00^{c}
Streptomycin	51.22^{a}	48.91^{a}
Aqueous extracts	33.36^{b}	36.40^{b}

Note: Means with the same superscript in the column are insignificantly different from each other at 5% level of significance.

The aqueous extracts of *P. sanguineus* was assessed to determine the antibacterial property against gram negative and gram positive bacteria (*E. coli and S. aureus*) using disc diffusion method. The data presented on Table 2 shows that *P. sanguineus* is relatively an effective antibacterial agent of *E. coli* and *S. aureus.* Aqueous extracts of *P. sanguineus* exhibited higher zone of inhibition against *E. coli* with 36.4mm than *S. aureus* with 33.36 mm. However, Fidalgo (1965) stated that *Cinnabarine,* a pigment produce by *Polyporus* was more active against Gram-positive than against Gram-negative bacteria.

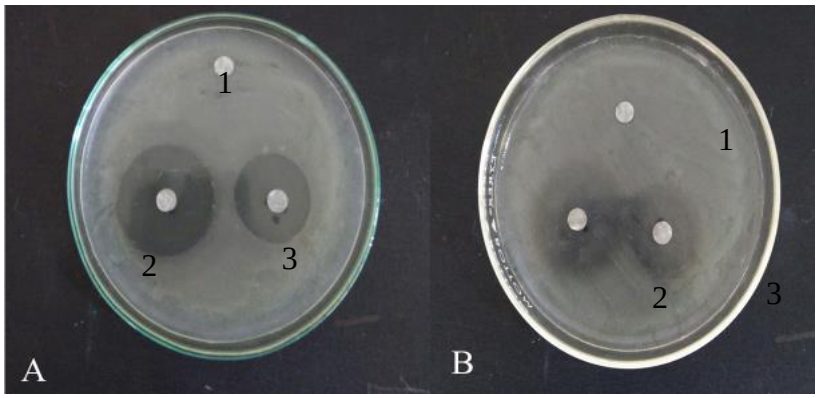

Figure 2. Zone of inhibition of *P. sanguineus* against *S. aureus* and *E. coli*. (A. distilled water, B. Streptomycin, C. Aqueous extracts)

With the significant findings obtained from the present study, the mycelia produced by *P. sanguineus* in mung bean decoction (MBD) is an effective antibacterial agent against *E.coli* and *S.aureus.* According to Eggert (1997) *Pycnoporous cinnabarinus* also showed antimicrobial activity to *E. coli* and *S. aureus* due to the cinnabarine and cinnabarinic acid that is produced both by this fungi. The appearance of inhibition manifest the capability of mycelia produced by *P. sanguineus* in MBD to control the growth and proliferation of bacteria on the medium. It could be possible that the bioactive compound that has antimicrobial property cause lysis into the membrane of the bacteria or may have penetrated the bacterial cells and disrupted the metabolic processes inside the cell thus it is suggested that myco-chemical components such as tannins, alkaloids, saponins and cardiac glycosides must also be evaluated.

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