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Preliminary Investigation on the Pharmacological Properties of Wood-Rotting Mushrooms Collected from Isabela State University, Echague, Isabela, Philippines

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Three wood-rotting fungi collected and isolated from Isabela State University, Echague, Isabela, Philippines were assessed to determine their mycochemical composition and antibacterial activity. Dried fruiting bodies were subjected to aqueous extraction prior extraction of their mycochemicals. Five (5) mycochemicals were screened including alkaloids, tannins, flavonoids, saponins and cardiac glycosides. Fruiting bodies of *G. lucidum* and *T. hirsuta* had the most number of mycochemicals detected which varied qualitatively from traceable to appreciable amount. Antibacterial property from the aqueous and acetonitrile extracts of the mushrooms was also evaluated following the standard disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. The antibacterial assay shows that all of the fungi exhibited a good source of antibacterial properties.

Keywords: *Ganoderma lucidum, Auricularia fuscosuccinea, Trametes hirsuta*, mycochemical analysis, antibacterial

Introduction

The decay of wood is brought about by the activities of wood-rotting fungi. These are distinct from other macrofungi as they have the ability to decompose lignin, a complex and structural polymer that occurs in woody cell walls of plants, without degrading extensive cellulose (Blanchette, 1991).

In the Philippines, wood decaying fungi such as *Ganoderma, Lentinus,Pleurotus* and others are grown in the wild or cultivated as source of food and pharmaceutical potential. They possess secondary metabolites that have evidently proven to have antioxidant, antidiabetic, antiflammatory, antitumor and anticancer properties (Acharya *et al*, 2015). However, there is limited or no information regarding these wild wood-rotting fungi found in Isabela province.

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This study highlighted the mycochemicals present in the aqueous extract of the three specimens and to screen the aqueous and acetonitrile extracts of each for their antibacterial activities.

Materials and Methods

Collection and Extraction of Samples

The fruiting bodies of the three wood-rotting fungi were obtained from decaying logs in Isabela State University, Echague, Isabela, Philippines. Acetonitrile and aqueous solvents were used in the extraction procedure of Dulay *et al*. (2017) with modifications. A total of 50 grams of air-dried fruiting bodies of each mushroom was extracted in 500mL of each solvent. Extracts were filtered using Whatman filter no. 1 and were subjected to reflux method.

Mycochemical Screening Test

Qualitative mycochemical test for the screening and identification of bioactive constituents was carried out in aqueous extracts of each of fungi using standard protocol of Sofowara (1993) as cited and modified by Jacob and David (2016). Results were determined based on the color and intensity of the reaction and interpreted as (+) if chemical is present in traceable amount, (++) if chemical is present in appreciable amount and (-) if chemical is absent.

Test for alkaloids

Extracts were dissolved individually in diluted hydrochloric acid and filtered. Filtrates were treated with solution of iodine in potassium iodide. Formation of a reddish brown colored precipitate indicated the presence of alkaloids.

Test for saponins

10ml of the filtered sample was mixed with 5mL of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and for the formation of emulsion which indicated the presence of saponins.

Test for tannins

Tannins were determined by boiling 0.5g of powdered sample in 20ml distilled water in a test tube and filtered. 0.1% $FeCl_3$ was added to the filtered samples and observed for brownish to green or a blue to black coloration which showed the presence of tannins.

Test for glycosides

Test for glycosides was determined by preparing 1mlof concentrated H_2SO_4in a test tube where 5 ml of aqueous extract from the sample was mixed with 2mL of glacial acetic acid containing 1 drop of $FeCl_3$. The above mixture was carefully added to 1ml of concentrated H_2SO_4so that it is underneath the mixture. In presence of cardiac glycoside in the sample, a brown ring would appear indicating the availability of the cardiac glycoside constituent.

Antibacterial Assay

The antibacterial properties of the extracts from the three mushrooms were determined by paper disc diffusion of Bauer *et al* (1996). *S. aureus* and *E. coli* was spread using a sterile cotton swab on Mueller Hinton Agar plate. Five millimeter diameter paper discs were impregnated with mushroom extract, and distilled water and Cotrimoxazole as standard and were placed equidistantly on the medium. Plates were incubated at 37 ℃ for 24hours.

Results and Discussion

All of the three wood decaying fungi, specifically *G. lucidum*, *A. fuscosuccinea* and *T. hirsuta* , are considered white rot fungi that degrade lignin and cellulose in cell walls resulting to bleaching in woody plants.

Mycochemical Analysis Test

Aqueous extract of each of the fungi were subjected to preliminary test of mycochemical analysis to detect presence of organic constituents including alkaloids, saponins, flavonoids, tannins and glycosides.

Table 1. Results of the mycochemical analyses on the three wood-rotting fungi using test solvent extracts

Bioactive Component	**Aqueous Extract**		
	G. lucidum	*A. fuscosuccinea*	*T. hirsute*
Alkaloids	++	++	+
Saponins	+	-	++
Flavonoids	-	-	+
Tannins	++	++	-
Glycosides	++	++	+

Notes: (-) absent, (+) traceable amount and (++) appreciable amount

Results shown in Table 1 revealed that all bioactive components varying from traceable to appreciable amount are present in *G. lucidum*

except for flavonoids. This is in contrast with the study of Paul *et al*. (2014) which reported *G. lucidum* to contain moderate amount of flavonoids. Presence of saponins in aqueous extract conforms to the study of Dandapat and Sinha (2015), wherein *G. lucidum* tested negative for saponins in all solvents except for aqueous. Moreover, all are present in *T. hirsuta* except for tannins. However, *A. fuscosuccinea* showed appreciable amount for alkaloids, tannins and glycosides.

Antibacterial Assay

Aqueous and acetonitrile extract of the three wood rotting fungi were assessed for their antibacterial assay against *S. aureus* and *E. coli* using disc diffusion method.

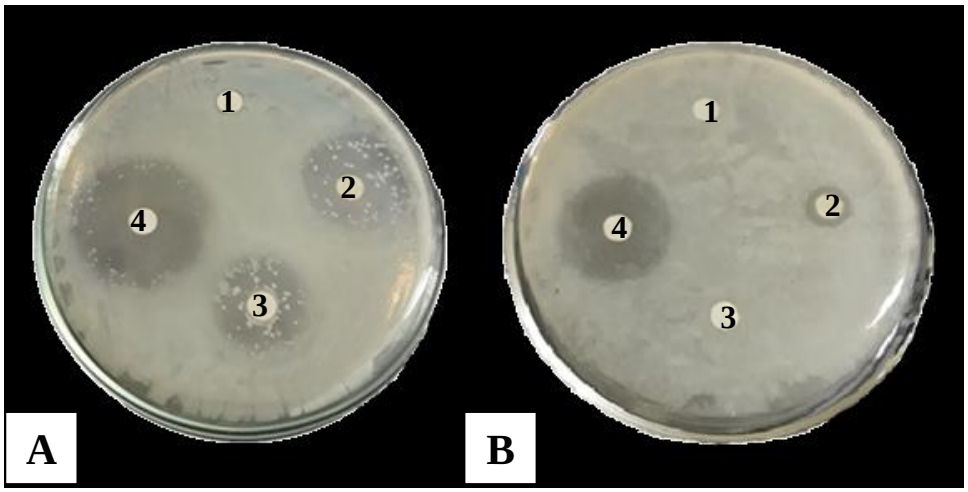

Figure 1. Result of antibacterial assay of *A. fuscosuccinea* against **(A)** *E. coli* **(B)** *S. aureus* (1. Distilled water, 2. Aqueous extract, 3. Aceto-nitrile extracts, 4. Cotrimoxazole)

Results of antibacterial assay of the extracts of three wood rotting fungi against *E.coli* and *S. aureus* are summarized in Table 1. After 24 hours of observation, it was found out that gram negative *E. coli* showed higher susceptibility to aqueous and acetonitrile extracts of the three wood decay fungi than gram positive bacteria *S. aureus*. Acetonitrile and aqueous extracts of *T. hirsuta* has the highest zone of inhibition with 28.19mm and 26.36mm among the treatments. Results from this segment was supported by Wu *et al* (2013) which reported that the presence of flavonoids in the extract display strong antibacterial activity against some of the bacteria especially *E. coli*. However, aqueous extract of *G. lucidum* exhibited the lowest inhibition among the treatments with 17.84 mm. Interestingly, no zones of inhibition were observed for the acetonitirile extract of *T. hirsuta* and *A. fuscosuccinea* against *S. aureus*. The highest inhibition observed

against *S. aureus* was aqueous extracts of *A. fuscosuccinea* with 13.50mm and *T. hirsuta* with 13.87mm.

Table 2. Zones of inhibition exhibited by aqueous and acetonitrile extracts of three wood-rotting fungi against *E. coli* and *S. aureus* after 24 hours

Treatments	Zone of Inhibition (mm)	
	S. aureus	*E. coli*
G. lucidum		
Distilled water	5.00^{c}	5.00^{d}
Aqueous extract	6.77^{c}	17.84^{c}
Acetonitrile extract	8.51^{b}	24.12^{b}
Cotrimoxazole	29.60^{a}	32.28^{a}
A. fuscosuccinea		
Distilled water	5.00^{c}	5.00^{c}
Aqueous extract	13.50^{b}	22.98^{b}
Acetonitrile extract	5.00^{c}	22.41^{b}
Cotrimoxazole	33.54^{a}	32.00^{a}
T. hirsuta		
Distilled water	5.00^{c}	5.00^{c}
Aqueous extract	13.87^{b}	26.36^{b}
Acetonitrile extract	5.00^{c}	28.19^{b}
Cotrimoxazole	29.52^{a}	38.25^{a}

Notes: Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance

Therefore, the result of this study highlighted the possible use of the three wood rotting fungi as source of natural bioactive components that can inhibit the growth of pathogenic microbes. This also suggests the potential of the three macrofungi may be dependent on the extraction solvent used and the bioactive constituents being extracted.

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