Research Article

Antibacterial property of *Auricularia polytricha* Mont. and *Trametes versicolor* Linn.

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Received: 3 January 2019 Revised: 18 February 2019 Accepted: 24 February 2019

Abstract

Objective: This study was aimed to determine the antibacterial properties of *A. polytricha* (Mont.) and *T. versicolor* (Linn.). **Material and methods:** Specifically, this study tried to find out if there is a significant difference using the extracts of *A. polytricha* (Mont.) and *T. versicolor* (Linn.) compared to positive controls specifically, Cefalexin® and Clindamycin® against *Staphylococcus aureus* and *Escherichia coli.* Tests for the confirmation for the presence of alkaloid, cardiac glycoside, cholesterol, flavonoid, saponin, steroid, tannin, and terpenoid were also included in the study to identify the presence of secondary metabolites in the *A. polytricha* (Mont.) and *T. versicolor* (Linn.) extract. Determination of antibacterial effect was done using the paper-disc method. By measuring the clear zone of inhibition, you can conclude if the extract of *A. polytricha* (Mont.) and *T. versicolor* (Linn.) have significant antibacterial effect against *Staphylococcus aureus* and *Eschirichia coli.* **Results and conclusion:** Results showed that extract of *A. polytricha* (Mont.) contains cardiac glycoside, steroids and tannins while the extract of *Trametes versicolor* (Linn.) contains alkaloids and steroids. In the determination for antibacterial effect, *Auricularia polytricha* (Mont.) and *Trametes versicolor* (Linn.) extract showed more effective result against *Staphylococcus aureus* and *Escherichia coli* compared to *Trametes versicolor* (Linn.) extract. They can be used as a natural, convenient yet effective alternative in the prevention of diseases caused by *Staphylococcus aureus* and *Eschirichia coli.*

***Keywords*:** *Trametes versicolor* (Linn.), *Auricularia polytricha* Mont., antibacterial

Introduction

Mushrooms have a great potential for the production of useful bioactive metabolites and that they are prolific resource for drug. Mushrooms characteristically contain a tremendous, variety of secondary metabolites that display a broad range of biological activities and the content and bioactivity of these compounds depend on how much the mushroom is prepared and consumed. The vast structural diversity of natural compounds found in mushroom, provide potential opportunities for discovering new drugs that rationally target the abnormal molecular and biochemical signals leading to cancer. Experience from ethnomedicine and extensive basic laboratory findings have shown that mushrooms could play an important role in the prevention and treatment of cancer (Russo et al., 2006).

The use of natural resource in the drug discovery has received much attention nowadays, not only for their potential as source of drugs, but also because they are natural, non-synthetic, and safe and their appreciation by consumers are very favorable. In fact, they have been the most successful source of drug leads for many years. The natural sources usually have a biological or pharmacological activity for use in pharmaceutical drug discovery and drug design. Between 1983 and 1994, 39% of antibacterial and anti-infective drugs were derived from natural products. Also, in that same time period, 39% of all new approved drugs were from either natural products or derived from natural products (http://www.lifepharms.com/).

In Asian countries, edible mushrooms have been valued as functional foods and as medicine resource due to their

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DOI: https://doi.org/10.31024/apj.2019.4.1.5

antioxidative, preventive, and therapeutic properties. A variety of secondary metabolites that includes phenolics, polyketides, terpenes, and other steroids are accumulated in mushroom. The phenolic compounds in Shiitake mushrooms *(Lentinusedodes)* and straw mushroom *(Volvariellavolvacea)* are believed to have contributed to their ability to scavenge free radicals, chelate metals, and inhibit lipoxygenase. Mushrooms can be used as natural oxidants in the forms of extracts, concentrates, powders or dietary supplements (Yim and Chye, 2010)

For many years, mankind has benefited from plants as natural source of drugs and herbal remedies. In fact, may studies have been focused on developing biological and pharmaceutical activity in plants, but attempts to explore mushrooms in such a way is much neglected. Hence, studies should be made to explore the potential use of mushrooms and their metabolites for treatment of a variety of human ailments.

Mushrooms, similar to plants, have a great potential for the production of useful bioactive metabolites and that they are prolific resource for drugs. Mushrooms characteristically contain a tremendous, variety of secondary metabolites that display a broad range of biological activities and the content and bioactivity of these compounds depends on how the mushroom is prepared and consumed. The vast structural diversity of natural compounds found in mushrooms, provide potential opportunities for discovering new drugs that rationally target the abnormal molecular and biochemical signals leading to fatal diseases especially cancer. Experience from ethnomedicine and extensive basic laboratory findings have shown that mushrooms could play an important role in prevention and treatment of cancer (Russo et al*.,* 2006).

In the present study, *Auricularia polytricha* (Mont.) and *Trametes versicolor* (Linn.) were to be evaluated for its anti-bacterial properties since not many studies had been done on the antibacterial properties of this species. Also, these mushrooms were to be evaluated for the presence of secondary metabolites which may lead to new findings of pharmacologically important substances. However, published studies on these mushrooms are quite limited. So the biological activities of *Auricularia polytricha* (Mont.) and *Trametes versicolor* (Linn.) metabolites need to be explored for its use as a source of drugs and functional food to contribute to new therapeutic effects and for the treatment of a variety of human ailments. Hence, this study is to be conducted to provide useful information about the said mushrooms based on the results of this study.

Materials and methods

Collection and authentication of mushroom

A. polytricha (Mont.) and *T. versicolor* (Linn.) were collected in places where there is an abundant supply of this species of mushrooms. Pictures, using a camera, were taken of each specimen directly from the place where it was located. In picking the specimen, a knife is very useful in cutting its base. Specimen was handled carefully to avoid damage. After gathering the collected *A. polytricha* (Mont.) and *T. versicolor* (Linn.), it was placed in a closed container so that the important features will not be lost or dry out.

Preparation and extraction of extracts

About 150 grams of freshly collected *A. polytricha* (Mont) and *T. versicolor* (Linn.) were segregated and was washed with distilled water. It was grinded using a blender. The 150 grams of each specimen was mixed with a solvent, specifically ethyl acetate ($CH_3COOCH_2CH_3$) and was placed for 24 hours in an oven where it has the standard room temperature (32ºC). The ratio of the grinded mushroom against the solvent is 1:2 or for every 1 gram of mushroom, its counterpart was 2 mL of the solvent.

Distillation process was needed to get the pure mushroom extract from the mixture of the solvent and the grinded mushroom. Distillation was used to purify the compound by separating it from a non-volatile or less-volatile material. Due to the difference in their boiling point, the compound which has the lower boiling point was separated first from the mixture. The compound which was retained in the distilling flask is said to be a less volatile material or the pure extract of a substance.

The solvent that was used to get the pure extract of *A. polytricha* (Mont.) and *T. versicolor* (Linn.) was ethyl acetate ($CH_3COOCH_2CH_3$). The liquid part of the mixture was filtered and transferred in a distilling flask which was heated for around 78ºC - 79ºC. The said temperature is the boiling point of ethyl acetate which belongs to the family of esters. When the temperature exceeds 79ºC, the ethyl acetate evaporates and the pure extract is retained in the beaker.

Phytochemical screening of Mushroom (Guevara, 2005)

There was only one (1) level of concentration of *A. polytricha* (Mont.) and *T. versicolor* (Linn.) extract which were used in phytochemical screening

100% Concentration = 25 mL pure Mushroom extract

Test for the presence of Alkaloid

In the test, Dragendorff's reagent and Mayer's reagent were used in determining the presence of alkaloid. A positive result indicates the presence of orange precipitate in Dragendorff's reagent and white precipitate with the Mayer's reagent.

For every 2 mL of the extract, add 1 mL HCl and 6 drops of

Mayer's reagent and Dragendorff's reagent. Any organic precipitate indicates the presence of alkaloids in the sample.

Test for the presence of Cardiac glycoside

For every 5 mL extract, treat it with 2 mL of glacial acetic acid containing one drop of Ferric chloride solution. This was underplayed with 1 mL of concentrated Sulfuric acid. A brown ring of the interface indicated deoxysugar characteristics of cardenolides. A violet ring might appear below the brown ring whereas acid layer, a greenish ring might form just gradually throughout thin layer.

Test for the presence of Cholesterol

2 mL of the extract and 2 mL of chloroform was added in a dry test tube. Then 10 drops of acetic anhydrides and 2 to 3 drops of concentrated sulfuric acid were added. A red color change to blue green color indicates the presence of cholesterol.

Test for the presence of Flavonoid

For the test of the presence of Flavonoid, Shinoda's test was followed. Few drops of concentrated HCl and Magnesium fillings were added in 1 mL of ethanol extract. Appearance of pink or magenta red color indicates the presence of flavonoids.

Test for the presence of Saponin

2 mL extract with 20 mL of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm of foam indicates the presense of saponin.

Test for the presence of Steroids

2 mL of acetic anhydride was added to 0.5 grams of ethanolic extract of each sample with 2 mL of sulfuric acid. The color change from violet to blue or green indicates the presence of steroids.

Test for the presence of Tannin

The Gelatin test was used to determine the presence of tannin. The formation of the jelly-precipitate indicates the presence of tannin. First, take an equivalent of 2 mL of mushroom extract from the stock extract and evaporate this to incipient dryness over a steam bath. Then extract the residue with 2 mL of hot distilled water. Add 5 drops 10% sodium chloride solution then filter and divide the filtrate into three test tubes. Take one of the three test tubes as the control; take an aqueous solution of tannic acid as reference standard. Take one portion of the filtrate of the mushroom extract with 3 drops of gelatin-salt reagent. Do likewise to the tannic acid solution, the reference standard. Compare with the control and the reference standard.

Test for the presence of Terpenoids

5 mL of extract was added to 2 mL of chloroform and 3 mL of concentrated Sulfuric acid to form a monolayer of reddish brown. Coloration of the interface will show to form positive result for the terpenoids.

Antibacterial Screening

Preparation of Culture Media

Suspend 19 grams of Mueller-Hinton Agar in 500 mL distilled water. Then boil the mixture to completely dissolve the medium. Sterilize it by using oven at 100ºC for 15 minutes. Then allow it to cool down about 50ºC and aseptically pour it to individual petri dishes. Allow it to solidify.

Preparation of Test Organism

The cultured *Staphylococcus aureus* and *Escherichia coli* was purchased in Philippine Applied Microbiology (PNCM), National Institute of Molecular Biology and Applied Microbiology (BIOTECH), University of the Philippines Los Baños (UPLB). It was incubated at 37ºC for 24 hours to revive and produce more colony growth.

Preparation of the Positive Control

Clindamycin® was used as positive control for *Staphylococcus aureus* (Gram positive bacteria) and Cefalexin® was used as positive control for *Escherichia coli* (Gram negative bacteria).

Pound a commercial tablet of an antibiotic (without removing it from its shelf). After turning it into a powder form, open the sachet and place the powdered tablet in a sterile petri dish. Add 1 mL distilled water to totally dissolve the powered tablet.

Preparation of Sensitivity Disc

Cut the Whattman filter paper no. 1 into round disk shapes using a puncher. The number of filter paper discs is dependent on the number of trials in the study. Place each labeled disks in petri dishes and sterilize at 121ºC for 15 minutes in an oven. After sterilizing those filter disks, soak them in the mushroom extract and commercial antibiotic for 1 hour.

Microbiological Technique

The determination of antibacterial property of the extracts was done using Pour-plate technique. Two sets of suspension of 19 grams Mueller-Hinton agar in 500 mL distilled water, one for *Staphylococcus aureus* and one for *Escherichia coli.* It was bring to boiled to completely dissolve the medium while stirring constantly. Allow it to cool about 50ºC.

Using sterile inoculating loops, inoculated cultured from

two different bacteria was diluted to each set of Mueller-Hilton agar. It was stirred to distribute bacteria so it can grow more colonies.

Assessment of Antibacterial Property

In determining the antibacterial property of the extract, paper disc technique was used. In each Mueller-Hinton agar treated with bacteria, it was carefully and aseptically impregnated with the soaked filter paper disks. The soaked filter paper discs were placed at the center of each agar plate to maximize the space for bacterial growth and also for the bacterial colony to grow more and be given an allowance for possible antibacterial activity which was demonstrated by producing a clear zone of inhibition around each disks. The plate was incubated at 37ºC for 24 hours. After the incubation, the plates were observed for any zone of inhibition (a clear zone) around the disk which indicates that growth of the organism had been inhibited by the chemical agent that diffuses into the agar from disks. Absence on inhibition indicates the resistance of the organism to the chemical agent present in the disk.

Results and discussion

Determinations of Secondary metabolites present in the extracts of *Auricularia polytricha* (Mont.) and *Trametes versicolor* (Linn.).

As shown in table 1, the secondary metabolites present in the extract of *A. polytricha* were cardiac glycoside, steroids and tannins. A violet ring was obtained which is an indication that cardiac glycoside was present in the extracts of *A. polytricha.* In the test for the presence of steroids, a color change from violet to green was observed. It indicates that *A. polytrichae* extract is positive for the presense of steroids. In the test for the presence of tannins, a formation of jelly-like precipitate was observed. It also indicates that *A. polytricha* extract is positive for that presence of tannins.

In *T. versicolor* extract, the secondary metabolites present are alkaloids and steroids. In the test for the presence of alkaloids, the formation of white precipitate using Mayer's reagent was observed. This means that the *T. versicolor* extract is positive for the presence of alkaloids. In the test for the presence of steroids, a color change to green was observed. This means that the *T. versicolor* extract is positive for the presence of steroids.

Table 1. Shows the results for phytochemical screening of *A. polytricha* and *T. versicolor* extract

Secondary Metabolites	*Auricularia polytricha* (Mont.)	*Trametes versicolor* (Linn.)
Alkaloids	Negative	Positive
Cardiac glycosides	Positive	Negative
Cholesterol	Negative	Negative
Flavonoids	Negative	Negative
Saponins	Negative	Negative
Steroids	Positive	Positive
Tannins	Positive	Negative
Terrpenoids	Negative	Negative

This implies that edible mushroom specifically *Auricularia polytricha* (Mont.) have more secondary metabolites present in their extracts compared to the non-edible mushrooms specifically *Trametes versicolor* (Linn.).

The effectiveness of *Auricularia polytricha* (Mont.) extract and *T. versicolor* (Linn.) extract in inhibiting the growth of *Staphylococcus aureas* and *Escherichia coli* is presented on table 2. As shown in the table, the *Auriculariapolytricha* (Mont.) extract and *T. versicolor* (Linn.) extract showed a good antibacterial against *Staphylcoccus aureas* and *Escherichia coli.*

The mean zone of inhibition of *A. polytricha* on *S. aureas* (gram-positive bacteria) was 5 mm while the mean zone of inhibition of *A. polytricha* on *E. coli* (gram-negative bacteria) is 7 mm. On the other, *T. versicolor* extract showed

Table 2. Shows the result of *Auricularia polytricha* (Mont.) extract and *T. versicolor* (Linn.) extract in inhibiting the growth of *S. aureus* and *E. coli*

Mushroom	*S. aureus*		*E. coli*		Computed t-value	Tabular t-value	Interpretation
	Mean	Variance	Mean	Variance			
A. polytricha (edible mushroom)	5	4.5	7	2	-1.94	2.776	Not significant
T. versicolor (non-edible mushroom)	2.5	1.5	4.67	8.67	-1.94	2.776	Not significant

a mean zone of inhibition of 2.5 mm on *S. aureus* (gram-positive bacteria) and a mean zone of inhibition of 4.67 mm on *E. coli* (gram-negative bacteria).

This implies that *A. polytricha* and *T. versicolor* showed a greater mean zone of inhibition on *E. coli* (gram-negative bacteria) and on *S. aureus* (gram-positive bacteria).

Comparison between the effectiveness of *Auricularia polytricha* (Mont.) extract against *Trametesversicolor* (Linn.) extract based on their antibacterial property in each microorganism

Staphylococcus aureus

As shown in table 3, the mean zone of inhibition of *A. polytricha* on *S. aureus* was 5 mm while the mean zone of inhibition of *T. versicolor* on *S. aureus* was 2.5 mm. It implies that *A. polytricha* has greater mean zone of inhibitions on *S. aureus* compared to *T. versicolor.*

In table 3, since the t-computed value of 2.51 is less than the t-tabular value of 2.776 at 0.05 level of significance with 4 degrees of freedom. This means that there is no significant difference between *A. polytricha extract* and *T. versicolor* extract based on its antibacterial action against *S. aureus.*

Escherichia coli

As shown in table 4, the mean zone of inhibition of *E. coli* was 7 mm while the mean zone of inhibition of *T. versicolor* on *E. coli* was 2.5 mm. It implies that *A. polytricha* has greater mean zone of inhibition of *E. coli* compared to *T. versicolor.*

In table 4, since the t-computed value of 1.75 is less than the t-tabular value of 2.776 at 0.05 level of significance with 4 degrees of freedom. This means that there is no significant difference between *A. polytricha* extract and *T. versicolor* extract based on its antibacterial action against *E. coli.*

Comparison between the effectiveness of *Auricularia polytricha* (Mont.) extract versus *Trametes versicolor* (Linn.) versus Clindamycin® for *S. aureus* and Cefalexin® for *E. coli* based on their antibacterial property.

Auricularia polytricha vs. *Trametes versicolor* (Linn.) vs. Clindamycin® on *S. aureus*

It is shown in tables 5 and 6 the level of significance among *Auriculariapolytricha* (Mont.) extract, *Trametes versicolor* (Linn.) extract and Positive control in inhibiting the growth of each microorganism used in the study.

In table 4 it shows that the F-computed value is 4.7 while the F-tabular value is 5.14 at 0.05 level of significance and with 2, 6 degrees of freedom. Since the computed value is less than the tabular value. This means that there is no significant difference between *A. polytricha, T. versicolor,* and Clindamycin® in inhibiting the growth of *S. aureus.*

Auricularia polytricha* vs. *Trametes versicolor* (Linn.) vs. Cefalexin® on *E. coli

In table 6 it shows that the F-computed value is 2.07 while the F-tabular value is 5.14 at 0.05 level of significance and with 2 and 6 degrees of freedom. Since the computed value is less than the tabular value. This means that there is no significant difference between *A. polytricha, T. versicolor* (Linn.) and Cefalexin® in inhibiting the growth of *E. coli.*

Table 3. Difference between *A. polytricha* and *T. versicolor* extract in inhibiting the growth of *S. aureus and E. coli*

Bacteria	*A. polytricha*		*T. versicolor*		Computed t-value	Tabular t-value	Interpretation
	Mean	Variance	Mean	Variance			
S. aureus	5	4.5	2.5	1.5	2.51	2.776	Not significant
E. coli	7	2	4.67	8.67	1.75	2.776	Not significant

Table 4. Difference between the *A. polytricha* extract, *T. versicolor* extract and Clindamycin® in inhibiting the growth of *S. aureus and E. coli*

Bacteria	*A. polytricha*		*T. versicolor*		Clindamycin®		Computed F-value	Tabular F-value	Interpretation
	Mean	Variance	Mean	Variance	Mean	Variance			
S. aureus	5	4.5	2.5	1.5	5	2	4.7	5.14	Not significant
E. coli	7	2	4.67	8.67	5.83	1.17	2.07	5.14	Not significant

Conclusion

The extract of *Auricularia polytricha* (Mont.) showed a positive result for the presence of cardiac glycosides, steroids and tannins. On the other hand, the extract of *Trametes versicolor* (Linn.) showed a positive result for the presence of alkaloids and steroids. Edible mushroom specifically *Auricularia polytricha* (Mont.) contains more secondary metabolites compared to non-edible mushroom specifically *Trametes versicolor* (Linn.). There is no significant relationship between the microorganisms used in the study specifically *Staphylococcus aureus* and *Escherichia coli* using *Auricularia polytricha* (Mont.) and also *Trametes versicolor* (Linn.) extract in terms of antibacterial action. Thus they can be used conveniently yet effective alternative in the prevention of diseases caused by *Staphylococcus aureus* and *Escherichia coli.*

Conflicts of interest

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