Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2020; 1(1): 44-49

www.microbiojournal.com
Received: 07-12-2019
Accepted: 11-01-2020

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Antibacterial property of *Pleurotus porrigens* Pers. and *Trametes versicolor* Linn. crude extracts against *Escherichia Coli* and *Staphylococcus aureus*

Jeffrey T Tonog and Manuela Cecille G Vicencio

Abstract
The study aimed to determine the significant difference between the *P. porrigens* Pers. crude extract and *T. versicolor* Linn. crude extract based on their antibacterial property on *S. aureus*.; determine the significant difference between the *P. porrigens* Pers. crude extract and *T. versicolor* Linn. crude extract based on their antibacterial property on *E. coli*; determine the significant difference between the following based on their antibacterial action: *P. porrigens* Pers. crude extract versus Clindamycin® on *S. aureus*; *T. versicolor* Linn. crude extract versus Clindamycin® on *S. aureus*; *P. porrigens* Pers. crude extract versus Cefalexin on *E. coli*; and to determine the significant difference between the following based on their antibacterial property: *Pleurotus porrigens* Pers. crude extract, *Trametes versicolor* Linn. crude extract, and Clindamycin® on *S. aureus*; and *Pleutorus porrigens Pers*. crude extract, *Trametes versicolor* Linn. crude extract, and Cefalexin on *E. coli.* The study revealed that the result of comparison of the bacteriostatic property of *P. porrigens* crude extract and *T. versicolor* crude extract on *S. aureus* using T-test is not significant since, the computed t-value of 2.434 is lower than the tabular t-value of 2.776. It implies that the antibacterial property of *P. porrigens* crude extract is comparable to *T. versicolor* crude extract against *S. aureus*; and the result of comparison of the bacteriostatic property of *P. porrigens* crude extract and *T. versicolor* crude extract on *E. coli* using T-test is not significant since, the computed t-value of 0.333 is lower than the tabular t-value of 2.776. It implies that the antibacterial property of *P. porrigens* crude extract is comparable to *T. versicolor* crude extract against *E. coli.* It implies that the antibacterial property of *P. porrigens* Pers. crude extract, *T. versicolor* Linn. crude extract is comparable to commercial antibacterial drugs used as positive control in the study against *S. aureus* and *E. coli*. It is recommended that same study must, use other species of edible and non-edible mushrooms to discover if they have antibacterial properties on the same kind of microorganisms used in the study, focus on the bactericidal property *P. porrigens* Pers. crude extract and *T. versicolor* Linn. crude extract, hence, bacteriostatic property was already used in the study and used other kind of microorganisms belong to the same kind of genus to determine the antibacterial property of *P. porrigens* Pers. extract and *T. versicolor* Linn. extract.

Keywords: *Pleurotus porrigens*, *Trametes versicolor* Linn, against *Staphylococcus aureus*

Introduction
Researchers in anti-infective are now desperately needed if a public if a public health crisis is to be averted. Infection of the multi-drug resistant isolates became more and more frequent, stimulating the search for new drugs with novel mechanism of actions. In contrast to bacterial infectious diseases, viral diseases cannot be treated by common antibiotics and specific drugs are urgently needed. The highly available choice in the market is the synthetic drugs. However, these non-natural drugs are not favorable to the consumers. Serious concerns on the carcinogenic properties and severe side effects that treatment of last resort for terminal diseases such as cancer. Therefore, there is great interest in finding new and safe drugs from natural sources.

The use of natural sources 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, safe and their appreciation by consumers are very favorable. In fact, they have consistently 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 anticancer drugs were derived from natural products. Also, in that same time, 39% of all new approved drugs were from either natural products or derived from natural products.

In some previous research, mushrooms have been reported to become an important source of novel bioactive compounds. It has a great potential as a nutritionally functional food and as a source for the development of drugs and nutraceuticals. It is estimated that approximately 50% of annual 5 million metric tons of cultivated edible mushrooms contain functional 'nutraceutical' or medicinal properties. The United States (US) Academy of science has defined functional foods as those that 'encompass potentially health products' including 'any modified food or food ingredient that may provide a health benefit beyond the nutrients it contains.

Asian countries are known to be rich sources of medicinal mushrooms. As a result of large numbers of scientific studies on medicinal mushrooms especially in Japan, China, and Korea, over the past three decades, many of the traditional uses have been confirmed and new applications developed. There are at least 270 species of mushrooms that have been identified to possess various therapeutic properties. The mushroom which has demonstrated such potential include *Auricularia (mu-er), Flammulina (enokitake), Grifola (maitake), Hericium, Lentinus (shiitake), Pleurotus (oyster), and Tremella (yiner)*. However, many of other potential mushrooms have not been thoroughly studied yet.

Mushrooms have long been appreciated for their flavor and texture. Now they are recognized as a nutritious food as well as an important source biologically active compounds of medicinal value (Breene, 1990) [6]. Mau *et al*. (2002) [16] had reported in their studies that medicinal mushrooms are commonly used for pharmaceutical purposes and as health foods. Hence, searching for new biological activities and other medicinal substances from mushrooms and to study the medicinal values of these mushrooms has become a matter of great significance.

Mushrooms are rich source of natural antibiotics. Antibiotic can be defined as a chemical substance produce by microorganisms (wholly or partly by chemical synthesis) which has the capacity to inhibit the growth of bacteria and even destroy bacteria and other microorganisms in dilute solution (Black, 2002) [4].

A research showed that the most significant antibiotics have been derived from fungi (Hardman *et al*., 2001) [11] such as penicillin, streptomycin, Chloramphenicol and vancomycin (Griffin, 1994) [10] where humans can benefit from the natural defensive strategies of the fungi that produce antibiotics to fight infection from microorganisms. Infections by multidrug resistant isolates of Candida spp., Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus spp., Enterococcus sp. and *Escherichia Coli*, among other became more and more frequent stimulating the search for new antibiotics with novel mechanisms of action (Kotra and Mobashery, 1998) [15].

In this study, *Pleutora porrigens* extract and *Trametes versicolor* Linn. were to be evaluated for its antibacterial properties. Published studies show that edible mushrooms are more useful than that of non-edible mushrooms as a source of delectable food. However, the study on non-edible mushrooms is too risky and limited for it will cost only danger to the people who do not know the proper way of preparing it. This study will try to find out which of this edible and non-edible mushroom has a greater potential in killing bacteria and its effectiveness compared with the given control.

Methodology

Pleurotus porrigens Pers. and *Trametes versicolor* Linn. were taken pictures for documentary purposes using digital camera directly from the place where they were are collected. In picking the specimen, a knife was used in cutting its base. Each specimen was handled carefully to avoid damage and destruction of any parts useful in identification or characterization of the specimen. The collected samples were placed in a closed separate container and are transported to the biology laboratory of the College of Science, University of Eastern Philippines, Catarman, Northern Samar, Philippines for identification and characterization purposes. The specimens were carefully identified by the adviser of the researcher.

About a large amount of freshly gathered *Pleurotus porrigens* Pers. and *Trametes versicolor* Linn. were segregated and washed separately with distilled water and air-dried. They were grinded separately using blender to extract the fluid material of the sample. *Pleurotus porrigens* Pers. and *Trametes versicolor* Linn. were extracted separately using extraction solvent ethyl acetate with ratio of 10 grams of grinded mushrooms into 20 mL of extraction solvent for 2 days at room temperature. After two days of soaking, they were filtered to separate the solid part from the extract using cheese cloth. Filtered extract where subjected to distillation procedure to separate the solvent from the crude extract.

Microbiological Screening

1. Source of Test Microorganism

The pure cultured Staphylococcus aureus and *Escherichia Coli* were bought from Philippine National Collection of Microorganism (PNCM) of National Institute of Molecular Biology and Applied Microbiology (Biotech), University of the Philippines Los Baños (UPLB). The test microorganism was transported to the College of Science, University of Eastern Philippines and was incubated for 24 hours at a temperature of 37 °C to keep the organism alive until it is used in experimental procedure.

2. Preparation of Nutrient Medium

38 grams of Mueller-Hinton agar was dissolved in 500 mL of distilled water. The mixture of Mueller-Hinton agar and distilled water were heated until simmer to completely dissolve the agar. The heated agar mixture where keep in liquid form by placing it a water at a temperature of 50°C.

3. Preparation of Sensitivity Discs

Whattman filter paper where cut using a metal puncher to create discs. The paper disc where wrap by a piece of bond paper and was sterilized in a hot air oven at 121°C for 15 minutes. The sterile paper disc was soaked in the crude extract of *Pleurotus porrigens* and *Trametes versicolor* and in commercial antibiotic solution used as the positive control in separate sterile petri dishes for 15 minutes.

4. Preparation of Positive Control Solution

The content of 250 mg capsule of commercial antibiotic Clindamycin® and Cefalexin® where dissolved in 1 mL distilled water in separate 25 mL beaker. 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).

5. Preparation of Petri Plate Culture

The 500 mL of prepared Mueller-Hinton nutrient medium were divided into two each about 250 mL. The first 250 mL of liquid nutrient medium was inoculated with S. aureus. During inoculation procedure a sterile inoculating loop was dipped in the pure culture of *E. coli* and transferred to 250 mL of liquid nutrient agar in a sterile Erlenmeyer flash. The inoculated liquid nutrient agar was poured to nine (9) sterile petri dishes and allowed to solidify. On the first three (3) sets petri plate containing solidified inoculated nutrient agar, paper disc soaked in crude extract *Pleurotus porrigens* was placed at the center of solidified media. In the next three sets, paper disc soaked in crude extract of *Trametes versicolor* was placed at the center of the petri plate. In the last three sets of petri plate paper disc soaked in clindamycin solution that serves as the positive control for S. aureus. The nine petri dishes were wrapped in piece of paper and were incubated in an inverted position for 24 hours at a temperature of 37 °C. Same procedure was done on the second 250 mL of liquid nutrient agar medium but this time *E. coli* were used as inoculums and Cefalexin as positive control.

6. Determination of Bacteriostatic Property

Twenty-four (24) hours after incubation petri plates were examined for bacteriostatic property of crude extract of *P. porrigens* Pers. and *T. versicolor* Linn. Bacteriostatic activity of the two extracts were determined by the zone of inhibition formed around the paper disc. The zone of inhibition is a clear zone around the disc resulting from the diffusion of substance from the disc into the culture medium. The zones of inhibitions are measured in millimeter (mm) using vernier caliper. Measurements of zone of inhibition were then recorded.

Results and Discussion

Table 1 shows the result of comparison of the bacteriostatic property of *P. porrigens* crude extract and *T. versicolor* crude extract on staphylococcus aureus using T-test. The table above reveals that *P. porrigens* crude extract has a mean zone of inhibition 5.1 mm and variance (sum of squares of mean zone of inhibition of *P. porrigens* crude extract) is 5.42. On the other hand, for *T. versicolor* crude extract the mean zone of inhibition is 2.5 mm with variance (sum of squares of zone of inhibition for *T. versicolor* crude extract) is 1.5. Computed t-value is 2.434 and the tabular t-value is 2.776. Since the computed t-value of 2.434 is lower than the tabular t-value of 2.776; therefore, the null hypotheses 1 that there is no significant difference in the antibacterial activity between *P. porrigens* crude extract and *T. versicolor* crude extract on *S. aureus* is accepted. It implies that the antibacterial property of *P. porrigens* crude extract is comparable to *T. versicolor* crude extract against *S. aureus*.

Table 1: Comparison of Bacteriostatic Property of *Pleurutos porrigens* Crude Extract and *Trametes versicolor* Crude Extract on *staphylococcus aureus*

Bacteria	*P. porrigens*		*T. versicolor*		Computed t-value	Tabular t-value	Interpretation
S. aureus	Mean	Variance	Mean	Variance	2.434	2.776	NS
	5.1 mm	5.42	2.5 mm	1.5			

Table 2 shows the result of comparison of the bacteriostatic property of *P. porrigens* crude extract and *T. versicolor* crude extract on *Escherichia Coli* using t-test. The table above reveals that *P. porrigens* crude extract has a mean zone of inhibition of 4.23 mm and variance (sum of squares of mean of inhibition of *P. porrigens* crude extract) is 1.89. on the otherhand, for *T. versicolor* crude extract the mean zone of inhibition for *T. versicolor* crude extract) is 8.67. Computed t-value is -0.333 and the tabular t-value is 2.776. Since the computed t-value of -0.333 is lower than the tabular t-value of 2.776; therefore, null hypothesis 2 that there is no significant difference in the antibacterial activity between *P. porrigens* crude extract and *T. versicolor* crude extract on *E. coli* is accepted. It implies that the antibacterial property of *P. porrigens* crude extract is comparable to *T. versicolor* crude extract against *E.coli*.

Table 2: Comparison of Bacteriostatic Property of *Pleurotus porrigens* Crude Extract and *Trametes versicolor* Crude extract on *Escherichia Coli*

Bacteria	*P. porrigens*		*T. versicolor*		Computed t-value	Tabular t-value	Interpretation
E. coli	Mean	Variance	Mean	Variance	-0.333	2.776	NS
	4.23 mm	1.89	4.67 mm	8.67			

Table 3a shows the result of comparison of the bacteriostatic property of *P. porrigens* and Clindamycin on *Staphylococcus aureus* using T-test. The table above reveals that *P. porrigens* crude extract has a mean zone of inhibition 5.1 mm and variance (sum of squares of mean zone inhibition of *P. porrigens* crude extract) is 5.42. On the other hand, for Clindamycin the mean zone of inhibition is 5 mm with variance (sum of squares of zone inhibition for Clindamycin®) is 2. Computed t-value is 0.090 and the tabular t-value of 2.776. Since the computed t-value of 0.090 is lower than the tabular t-value of 2.776; therefore, null hypothesis 3a that there is no significant difference in the antibacterial property between property between *P. porrigens* crude extract and commercial antibiotic drug Clindamycin® on *S. aureus* is accepted. It implies that the antibacterial property of *P. porrigens* crude extract is comparable to commercial antibacterial drug Clindamycin® against *S. aureus*.

Table 3a: Comparison of Bacteriostatic Property of *Pleurotus porrigens* Crude Extract and Clindamycin® on *Staphylococcus aureus*

Bacteria	*P. porrigens*		Clindamycin®		Computed t-value	Tabular t-value	Interpretation
S. aureus	Mean	Variance	Mean	Variance	0.090	2.776	NS
	5.1 mm	5.42	5 mm	2			

Table 3b shows the result of comparison of the bacteriostatic property of *Trametes versicolor* crude extract and Clindamycin® on *Staphylococcus aureus* using T-test. The above table reveals that *T. versicolor* crude extract has a mean zone of inhibition of 2.5 mm and variance (sum of squares of mean zone of inhibition for Clindamycin®) is 2. Computed t-value is -3.2899 and the tabular t-value of 2.776. Since the computed t-value of -3.2899 is lower than the tabular t-value of 2.776; therefore, null hypothesis 3b that there is no significant difference in the antibacterial activity between *T. versicolor* crude extract and commercial antibiotic drug Clindamycin® on *S. aureus* is accepted. It implies that the antibacterial activity of *T. versicolor* crude extract is comparable to commercial antibacterial drug Clindamycin® against S. aureus.

Table 3b: Comparison of Bacteriostatic Property of *Trametes versicolor* Crude Extract and Clindamycin® on *Staphylococcus aureus*

Bacteria	*T. versicolor Linn.*		Clindamycin®		Computed t-value	Tabular t-value	Interpretation
S. aureus	Mean	Variance	Mean	Variance	-3.2899	2.776	NS
	2.5 mm	1.5	5 mm	2			

Table 3d shows the result of comparison of the bacteriostatic property of *Trametes versicolor* crude extract and Cefalexin® on *Escherichia Coli* using T-test. The above table reveals that *T. versicolor* crude extract has a mean zone of inhibition of 4.67 mm and variance (sum of squares of mean zone of inhibition of *T. versicolor*) is 8.67. On the other hand, for Cefalexin® the mean zone of inhibition is 5.83 mm with variance (sum of squares of zone of inhibition for Cefalexin®) is 1.17. Computed t-value is -0.910 and the tabular t-value of 2.776. Since the computed t-value of -0.919 is lower than the tabular t-value of 2.776; therefore, the null hypotheses 3d that there is no significant difference in the antibacterial property between the *T. versicolor* crude extract and commercial antibiotic drug Cefalexin® on *E. coli* is accepted. It implies that the antibacterial activity of *T. versicolor* crude extract is comparable to commercial antibacterial drug Cefalexin® against *E. coli*.

Table 3d: Comparison of Bacteriostatic Property of *Trametes versicolor* Crude Extract and Cefalexin® on *Escherichia Coli*

Bacteria	*T. versicolor Linn.*		Cefalexin®		Computed t-value	Tabular t-value	Interpretation
E. coli	Mean	Variance	Mean	Variance	-0.910	2.776	NS
	4.67 mm	8.67	5.83 mm	1.17			

Table 4a shows the result of comparison of the bacteriostatic property of *Pleurotus porrigens*, *Trametes versicolor* crude extract and Clindamycin® on *Staphylococcus aureus* using F-test. The above table reveals that *P. porrigens* crude extract has a mean zone of inhibition of 5.1 mm with a variance of 5.42, *T. versicolor* crude extract has a mean zone of inhibition of 2.5 mm with a variance of 1.5 mm and Clindamycin® has a mean zone of inhibition of 5 mm with a variance with a variance of 2. Computed F-value is 4.37 and the tabular f-value of 5.14. Since the computed f-value of 4.37 is lower than the tabular f-value of 5.14; therefore, null hypotheses 4a that there is no significant difference in the antibacterial activity between *P. porrigens* crude extract, *T. versicolor* crude extract, and commercial antibiotic drug Clindamycin® on S. aureus is accepted. It implies that *P. porrigens* crude extract, *T. versicolor* crude extract, and commercial antibacterial drug Clindamycin® are comparable with each other in terms with their antibacterial property against *S. aureus*.

Table 4a: Comparison of Bacteriostatic Property of *Pleurotus porrigens* Crude extract, *Trametes versicolor* Crude Extract and Clindamycin® on *Staphylococcus aureus*

Bacteria	*P. porrigens*		*T. versicolor*		Clindamycin®		F-value		Interpretation
S. aureus	Mean	Variance	Mean	Variance	Mean	Variance	Computed	Tabular	NS
	5.1 mm	5.42	2.5 mm	1.5	5 mm	2	4.37	5.14	

Table 4b shows the result of comparison of the bacteriostatic property of *Pleurotus porrigens*, *Trametes versicolor* crude extract and Cefalexin® on *Escherichia Coli* using F-test. The above table reveals that *P. porrigens* crude extract has a mean zone of inhibition of 4.23 mm with a variance of 1.89, *T. versicolor* crude extract has a mean zone of inhibition of 4.67 mm with a variance of 8.67 and Cefalexin® has a mean zone of inhibition of 5.83 mm with a variance of 1.17. Computed f-value is 1.05 and the tabular f-value of 5.14. Since the computed f-value of 1.05 is lower than the tabular f-value of 5.14; therefore, null hypotheses 4b that there is no significant difference in the antibacterial activity between *P. porrigens crude extract*, *T. versicolor* crude extract and commercial antibiotic drug Cefalexin® on *E. coli* is accepted. It implies that *P. porrigens* crude extract, *T. versicolor* crude extract and commercial antibacterial drug Cefalexin® are comparable with each other in terms with their antibacterial property against *E. coli*.

Table 4b: Comparison of Bacteriostatic Property of *Pleurotus porrigens* Crude extract, *Trametes versicolor* Crude Extract and Cefalexin® on *Escherichia Coli*

Bacteria	*P. porrigens*		*T. versicolor*		Cefalexin®		F-value		Interpretation
E. coli	Mean	Variance	Mean	Variance	Mean	Variance	Computed	Tabular	NS
	4.23 mm	1.89	4.67 mm	8.67	5.83 mm	1.17	1.05	5.14	

Conclusion

The result of comparison of the bacteriostatic property of *P. porrigens* crude extract and *T. versicolor crude* extract on *S. aureus* using T-test revealed that they are not significant. Since the computed t-value of 2.434 is lower than its tabular t-value of 2.776. It implies that the antibacterial property of *P. porrigens* crude extract is comparable to *T. versicolor* crude extract against *S. aureus*. The result of comparison of the bacteriostatic property of *P. porrigens* crude extract and *T. versicolor* crude extract on *E. coli* using T-test revealed that they are not significant. Since the computed t-value of -0.333 is lower than the tabular t-value of 2.776. It implies that the antibacterial property of *P. porrigens* crude extract is comparable to *T. versicolor* crude extract against *E. coli*.

In the determination of significant difference of the following, statistical study revealed that are all not significant: 3a) the result of comparison of the bacteriostatic property of *P. porrigens* and Clindamycin on *S. aureus* using T-test is not significant. Since the computed t-value of 0.090 is lower than the tabular t-value of 2.776. It implies that the antibacterial drug Clindamycin® against *S. aureus*; 3b) the result of comparison of the bacteriostatic property of *T. versicolor* crude extract and Clindamycin® on *S. aureus* using T-test is not significant. Since the computed t-value of -3.2899 is lower than the tabular t-value of 2.776. It implies that the antibacterial activity of *T. versicolor* crude extract is comparable to commercial antibacterial drug Clindamtcin® against *S. aureus*; 3c) the result of comparison of the bacteriostatic property of *P. porrigens* crude extract and Cefalexin® *on E. coli* using T-test is not significant. Since the computed t-value of -2.25 is lower than the tabular t-value of 2.776. It implies that the antibacterial drug Cefalexin® against *E. coli*; and 3d) the result of comparison of the bacteriostatic property of *T. versicolor* crude extract and Cefalexin® on *E. coli* using T-test is not significant. Since the computed t-value of 0.910 is lower than the tabular of 2.776. It implies that the activity of *T. versicolor* crude extract is comparable to commercial antibacterial drug Cefalexin® against *E. coli*.

In comparison of the bacteriostatic property of the following, it was also revealed that they are not significant: 4a) *P. porrigens*, *T. versicolor* crude extract, and Clindamycin® on *S. aureus* using F-test, it was revealed that they are not significant. Since the computed f-value of 4.37 is lower than the tabular f-value of 5.14. It implies that the *P. porrigens* crude extract, *T. versicolor* crude extract, and commercial antibacterial drug Clindamycin® are comparable with each other in terms with their antibacterial property against *S. aureus*; and 4b) in the comparison of result of the bacteriostatic property of *P. porrigens* crude extract, *T. versicolor* crude extract, and Cefalexin® on *E. coli* using F-test, it was revealed that they are not significant. Since the computed f-value of 1.05 is lower than the tabular f-value of 5.14. It implies that *P. porrigens* crude extract, *T. versicolor* crude extract, and commercial antibacterial drug Cefalexin® are comparable with each other in terms with their antibacterial property against *E. coli*.

Acknowledgement

Not available

Author's Contribution

Not available

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article
JT Tonog, Manuela Cecille G Vicencio. Antibacterial property of *Pleurotus porrigens* Pers. and *Trametes versicolor* Linn. crude extracts against *Escherichia Coli* and *Staphylococcus aureus*. Journal of Advances in Microbiology Research 2020; 1(1): 44-49