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Myko-actives and functional activities of Philippine wild mushroom *Trametes elegans*

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Abstract

Trametes elegans (Family Polyporaceae), is a wood-rot mushroom which usually grows on decaying hard woods, logs and branches from June to November. In order to establish its nutraceutical potential, the mycochemical composition, total phenolic content, antioxidant, antibacterial, and cytotoxic activities were determined in this study. The mycochemical screening revealed the presence of essential oil, fatty acids, anthraquinones, anthrones, tannins, flavonoids, phenols, alkaloids, steroids and coumarins. *T. elegans* ethanolic extract exhibited radical scavenging activity (57.56%) and contained total phenolics of 27.59 mg AAE/g sample. The extract also showed inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* with the diameter zones of inhibition of 8.30 mm and 8.07 mm, respectively. The cytotoxicity assay revealed that *T. elegans* is considered highly toxic to brine shrimp nauplii with an LC_{50} value of 32.57 µg/ml. Thus, wild fruiting bodies of *T. elegans* contain bioactive compounds with functional activities such as antibacterial and antioxidant.

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Introduction

Mushrooms are macro fungi with distinctive fruiting body, which can be hypogenous or epigeous, large enough to be seen with the naked eye and to be picked by hand (Chang and Miles, 1992).

They have been traditionally believed to be remedies for many diseases (De Silva *et al.*, 2013) and are known to be prolific producers of bioactive metabolites (Wasser, 2011). Both the fruiting body and mycelia of different mushrooms contain various compounds such as terpenoids, steroids, polyphenol, polyketides, polyglucan, flavonoids, alkaloids, polysaccharides and dietary fibers which exert several pharmacological activities.

The polysaccharides obtained from mushrooms are considered to be compounds capable of modulating the immune response in animals and humans and inhibiting the growth of certain tumours (Lindequist *et al.*, 2005).

Trametes elegans is a large white, thick fleshed, fan-shaped pileus; and its pores are mixture of circular and maze-like in appearance. Usually their stipe are absent or stublike attach on decaying hardwoods. They usually grow on decaying hardwoods, stump, logs and branches, from June to November. Among *Trametes* species, *T. versicolor* is the most popular and commonly utilized. Teoh and Mashitah (2012) reported that *T. versicolor* can be a source of antifungal agents against wood-decaying fungi present in the rubberwood. *Trametes* mushrooms exhibit antibacterial, antitumor and anti-inflammatory activities (Wasser and Weis, 1999). Moreover, previous works reported that *Trametes* is among the most versatile white-rotters with potential in bioremediation application (Nyanhongo *et al.*, 2007).

This present work evaluated the bioactive compounds and functional activities particularly on the antioxidant and antibacterial properties of wild fruiting bodies of *T. elegans* in order to establish its role in pharmaceutical industry.

Materials and method

Source of mushroom fruiting bodies

The wild fruiting bodies of *T. elegans* were collected in the Lingap Kalikasan Park of Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. The fruiting bodies were cut into small pieces and air-dried for four days. Samples were pulverized using a food blender. This sample was extracted and subjected to mycochemical screening and biological activity evaluation.

Ethanol extraction

The bioactive compounds of the air-dried mushroom were extracted following the procedure of Wong *et al.* (2013) with minor modifications. Twenty grams of powdered sample were soaked in 200 ml of ethanol. The mixture was placed in an Erlenmeyer flask covered and wrapped with an aluminium foil and was allowed to stand in the dark for 48 hours at room temperature. The extract was separated from the residue by filtration through Whatman filter paper No. 1. The ethanol was removed under reduced pressure at 40°C-45°C using a rotary evaporator up to dryness and then the extract was obtained and kept in a clean vial, closed tightly and stored in a cool dry place.

Mycochemical screening

The mycochemical screening of the fruiting bodies were carried out following the procedures described by Guevara *et al.* (2005).

Radical scavenging activity assay

The concentrated extract was used to make a stock solution and aliquot was taken to make 1000 µg/ml dilution and 1000 µg/ml cathechin as control. One ml of the prepared stock solution was mixed with 4 ml of 0.1 mM DPPH solution in separate plastic cuvette. Reaction was done in triplicate. The prepared mixtures were incubated in the dark at 37°C for 30 min. The absorbance readings were monitored at 517 nm using a UV VIS spectrophotometer. The ability to scavenge the DPPH radical was calculated using the formula: % Radical Scavenging Effect = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

Estimation of total phenolic content

The amount of total phenolics in the extract was determined using Folin-Ciocalteu method (Sunita and Dhananjay, 2010). Ascorbic acid was used as a standard and the total phenolics were expressed as mg/g Ascorbic Acid Equivalents (AAE).

The different concentrations of ascorbic acid and 1 mg/ml concentration of mushroom extract were prepared in methanol. Each sample (0.5 ml) was introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 min at room temperature prior to absorbance reading at 760 nm spectrophotometric ally. All tests were performed in triplicate.

Determination of antibacterial property

The antibacterial activity of the extract of the fruiting bodies of *T. elegans* was determined following the paper disc diffusion method of Bauer *et al.* (1996). *Staphylococcus aureus* and *Escherichia coli* were cultured in 9 ml of nutrient broth (NB) medium and incubated at 37 °C. After 24 h, the turbidity of each bacterial culture was adjusted to equal that of 0.5 McFarland standard, which approximated 1.5×10^8 cells/ml. The bacterial suspension was spread using a sterile cotton swab on Mueller Hinton agar plate. Six millimetre diameter paper discs impregnated with 20 µL of 100 mg/ml of mushroom extract dissolved in 95% ethanol and streptomycin as standard were placed equidistantly on the media with bacteria. Plates were incubated at 37 °C, and the zone of inhibition was measured after 24 h. Each test was done in triplicate.

Brine shrimp cytotoxicity assay

The toxic effect of mushroom extract was evaluated using brine shrimp cytotoxicity assay. Brine shrimp nauplii were exposed to the different concentrations of the extract (0 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml, and 1000 µg/ml). After 24 hours of exposure, percentage mortality was determined and the LC_{50} value was established using probit analysis.

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test (DMRT) at 5% level of significance.

Results and discussion

Mycochemical constituents of T. elegans

Mushrooms are medicinal primarily because they contain a number of biologically active compounds. The mycochemical constituents of wild fruiting bodies of *T. elegans* were investigated in the present work.

Table 1. Mycochemicals of wild fruiting bodies of *T. elegans*.

Mycochemicals	Result
Terpenoids	Not Detected
Flavonoids	Present
Tannins	Present
Phenols	Present
Steroids	Present
Cardiac glycosides	Not Detected
Alkaloids	Present
Anthraquinones	Present
Anthrones	Present
Coumarins	Present
Essential oil	Present
Fatty acids	Present
Saponins	Not Detected

Table 1 presents the results of mycochemical screening. Apparently, the *T. elegans* contained flavonoids, tannins, phenols, steroids, alkaloids, anthraquinones, anthrones, coumarins, essential oils, and fatty acids. However, terpenoids, cardiac glycosides, and saponins were not detected. Some mushrooms also contain the same mycochemicals. For instance, Kalaw and Albinto (2014) reported that alkaloids, flavonoids, saponins and terpenoids are present in both fruiting bodies of *Coprinus comatus* and *Pleurotus cyctidiosus*. In addition, Hoque *et al.* (2015) also reported that extracts of *Ganoderma lucidum* has alkaloids, terpenoids, carbohydrates, tannins, flavonoids, and steroids. Flavonoids have long been recognized to possess anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, anti-viral, and anti-carcinogenic activities (Tapas *et al.*, 2008). Lim *et al.* (2006) reported the antimicrobial activity of tannin extracted from *Rhizophora apiculata* bark. Patel *et al.* (2012) reported that alkaloid has various types of pharmacological activity such as antimicrobial, antifungal, antitumor, cytotoxic, antiplasmodial, antioxidant, antimutagenic, antigenotoxic and hallucinogenic properties. Coumarin has been shown to activate other cells of the immune system. They can be used not only to treat cancer but also to treat the side effects caused by radiotherapy (Agarwal, 2000).

Table 2. Radical scavenging activity and total phenolic content of *T. elegans* extract.

Treatment	Radical Scavenging Activity (%)	Total Phenolic Content (mg AAE /g)
T. elegans extract	57.56	27.59
Catechin (control)	50.15	---

Data presented as means (n = 3). The concentration of ethanol extract of the mushroom fruiting body used was at 1 mg/ml.

Antioxidant activity of T. elegans

Antioxidants play a vital role in the protection of human organism against free radicals that causes anemia, asthma, premature sign of aging and cancer (Buricova and Reblova, 2007). The antioxidant activity of *T. elegans* was evaluated using DPPH radical scavenging activity. As shown in Table 2, the extract exhibited radical scavenging activity with a mean of 57.56%, which means that the ethanol extract of *T. elegans* can inhibit free radicals. Similarly, other mushrooms also showed radical scavenging activity. For, instance, Dulay *et al.* (2016) reported that the mycelia of both *Volvariella volvacea* and *Schizophyllum commune* grown in different liquid culture media showed different radical scavenging activities against free radicals. According to Hoque *et al.* (2015) the scavenging effect of the extracts increases with the concentration. Methanol extract of *G. lucidum* (MEGL) showed the highest radical scavenging activity whereas pet ether extract (PEGL) showed the lowest activity. These findings suggest that the antioxidant activities of mushrooms are dependent to several factors.

Total phenolic content of T. elegans

The total phenolic content was also evaluated spectrophotometrically using the Folin-Ciocalteu method. *T. elegans* extract had a total phenolic content with of 27.59 mg AAE/g sample (Table 2). Phenolics are potent antioxidants (Shahidi and Wanasundara, 1992). These compounds can donate hydrogen to free radicals and this way to stop the chain reaction of lipid oxidation at the initial stage. This ability of phenolic compounds to scavenge radicals comes due to the presence of their phenolic hydroxyl groups (Sawa *et al.*, 1999). Furthermore, flavonoids are widely group of natural compounds and the most important natural phenolics. These compounds have a large number of biological and chemical activities including radical scavenging properties (Ghafar *et al.*, 2010).

Antibacterial activity of T. elegans

The antibacterial activity of the ethanolic extract of *T. elegans* was also evaluated in the present study. Table 3 shows the diameter zone of inhibition of ethanol extract of *T. elegans* against S. *aureus* and *E. coli*

after 24 hours of incubation. It can be seen that the extract showed inhibitory activity against both *S. aureus* and *E. coli* with diameter zones of inhibition of 8.07 mm and 8.30 mm, respectively. This suggests that *T. elegans* ethanol extract has a potential antibacterial agent. Similarly, the ethanolic extract of *Lentinus tigrinus* showed high antibacterial activity against *S. aureus* (Dulay *et al.*, 2014). Moreover, Kalaw and Albinto (2014) also reported the antibacterial activity of ethanol and acetone extracts of *Coprinus comatus* and *Pleurotus cystidiosus* against *S. aureus* but ineffective against *E. coli*. This difference on the inhibitory activity could be due to the unique chemical properties of each mushroom. Aside from this, antibacterial properties of mushrooms are also solvent dependent.

Table 3. Diameter of zone of inhibition of the ethanol extract of *T. elegans* against *S. aureus* and *E. coli*.

Treatment	Diameter zone of inhibition (mm)	
	S. aureus	*E. coli*
T. elegans extract	8.07 ± 0.65 [b]	8.30 ± 0.55 [b]
Streptomycin sulphate	23.37 ± 0.89 [a]	27.22 ± 6.57 [a]
Ethanol (solvent)	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]

Data presented as means ± SD (n = 3). Treatment means with the same letter of superscript are not significantly different from each other at 5% level of significance using DMRT. The concentration of ethanol extract of the mushroom fruiting body used was at 100 mg/ml in 95% ethanol.

Cytotoxic effect of T. elegans

Brine shrimp bioassay is considered as a quick preliminary screening for the presence or absence of bioactivity and also used to determine the cytotoxicity of crude extracts (Syahmi *et al.*, 2010). In this paper, the cytotoxic effect of *T. elegans* extract using brine shrimp nauplii is also reported (data not shown).

The percentage mortality of the nauplii was recorded after 24 hours. The 100% mortality was registered at 1000 µg/ml, followed by 100 µg/ml with 70.00% mortality.

The computed median lethal concentration (LC_{50}) value of *T. elegans* ethanol extract was 32.57 ug/ml. This LC_{50} value indicates that *T. elegans* is highly toxic based on the toxicity rating established by Clarkson *et al.* (2004).

The result suggests that the ethanol extract of *T. elegans* can be a source of toxic compounds which are potentially anticancer agents. Extracts of other mushrooms also showed cytotoxic effects. *Lactarius edulis* petroleum ether, dichloromethane and methanol extracts, and *T. letestui* ethanol extract exhibited mild cytotoxic activity (brine shrimp test; LC_{50}= 88, 69.6, 26.7 and 69.7 µg/ml, respectively) while *Agaricus sp.* aff. *arvensis* ethanol extract had the highest cytotoxic activity (LC_{50} = 19.90 µg/ml) (Baraza *et al.*, 2007). Nyigo *et al.* (2009) observed that the crude extracts of *Agaricus* and *Termitomyces* species exhibited significant activity in brine shrimp bioassay.

Conclusion

In conclusion, wild fruiting bodies of *T. elegans* have essential oil, fatty acids, anthraquinones, anthrones, tannins, flavonoids, phenols, alkaloids, steroids and coumarins. Ethanol extract of this wild mushroom exhibits radical scavenging and antibacterial activities, contains total phenolics, and shows cytotoxic effect in brine shrimp.

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