IJBPAS, June, 2018, 7(6): 966-975

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MYCOCHEMICALS, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *Polyporus grammocephalus* Berk (BIL7749)

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Received 23rd Feb. 2018; Revised 5th March 2018; Accepted 30th March 2018; Available online 1st June 2018

DOI: https://doi.org/10.31032/IJBPAS/2018/7.6.4455

ABSTRACT

Polyporus grammocephalus, from the family Polyporaceae, is a wood-rot mushroom that usually growing on trunks of trees, decaying wood and logs. In order to establish the nutraceutical potentialities of this mushroom, the mycochemical composition, antioxidant property, and cytotoxic effect of its fruiting bodies were evaluated. The mycochemical screening revealed that *P. grammocephalus* contains essential oils, phenols, triterpenes, steroids, fatty acids, sugars, anthraquinones, coumarins, anthrones, tannins, flavonoids, and alkaloids. However, terpenoids, cardiac glycosides, and saponins were not detected. *P. grammocephalus* ethanol extract exhibits DPPH radical scavenging activity (26.37%) and contains total phenolics (38.58 mg GAE/g). Brine shrimp toxicity assay revealed this mushroom is highly toxic with an LC50 value of 73.78µg/ml. Therefore, *P. grammocephalus* fruiting bodies contain bioactive compounds with enormous pharmacological activities.

Keywords: *Polyporus grammocephalus*, mycochemicals, DPPH radical scavenging, brine shrimp lethality assay.

INTRODUCTION

Mushrooms are known because of their high nutritional and medicinal values, possessing numerous bioactive substances including phenolic compounds, polyketides, terpenes,

and steroids **[1, 2, 3]**. Some of these compounds have remarkable importance to humanity displaying a wide range of useful antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, hepatoprotective, antidiabetic, hypolipedemic, antithrombotic and hypotensive activities owing to the mycochemicals that are present in the fruiting bodies **[4, 2]**. In the past years, the therapeutic value of mushrooms has come to the interest of researchers, solving problems posed by the use of synthetic drugs. Several antioxidants have been found and reported in the past few decades; however, reports of carcinogenicity of synthetic antioxidants have augmented the need to search for naturally occurring antioxidants **[3, 5]**. Mushrooms have phenolic compounds that are found to be excellent antioxidants **[6, 7]**.

Polyporus grammocephalus is a wild wood-rotting basidiomycetes belonging to Family Polyporaceae. It is naturally occurring mushroom in the different areas in the Philippines such as in Botolan Zambales, Nueva Vizcaya and Los Banos, Laguna **[8, 9, 10, 11]**. Based on the ethnomycological studies, this mushroom is consumed as food by the Aeta tribe and Gaddang community. Another wild strain of *P. grammocephalus* from Central Luzon State University, Science City of Muñoz, Nueva Ecija was successfully isolated and domesticated by Dulay and Rivera **[8]**. However, the functional activities must establish, hence this study.

A brine shrimp lethality assay is convenient method in determining the toxic effect of a specimen and is indispensable for the successful development of a pharmacological or cosmetic preparation **[12]**. Over the years, the utilization of cell-based assays by pharmaceutical companies for high- quantity and secondary chemical cytotoxicity screening has increased **[13]**. In order to study the toxicity of *P. grammocephalus*, brine shrimp lethality bioassay was performed because of its positive relationship with cytotoxic activity in some human solid tumors and with pesticidal activity **[14, 15, 16]**. This bioassay was proposed by Michael *et al.* **[14, 17]** and modified by others **[14, 18, 19]**. For the evaluation of the potential of *P. grammocephalus* as source of therapeutic drugs, this study also highlighted the mycochemical and antioxidant property of the mushroom including the radical scavenging activity and total phenolic content.

This present work elucidated the rich mycochemical compositions, antioxidant activity and cytotoxic effect of fruiting bodies of *P. grammocephalus*.

MATERIALS AND METHODS

Mushroom Sample

The fruiting bodies of *P. grammocephalus* grown in the best formulation reported by Dulay and Rivera **[8]** were air-dried and pulverized using a food blender. The sample was extracted and subjected to mycochemical screening and bioactivity assays.

Mycochemical Screening

The mycochemical compositions of mushroom were determined following the procedures of Guevara *et al*. **[20]**. The different mycochemicals were detected as spot in thin layer chromatography (TLC).

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the ethanol extract of mushroom was determined. A 100 µl of test sample in ethanol was mixed with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtitter plates. The mixture was vigorously shaken and left to stand for 30 minutes in the dark. The absorbance was measured and the inhibition of DPPH free radicals was calculated using the equation: Percentage scavenging effect = $[(A_{control} - A_{sample}) / A_{control}] \times 100$ where $A_{control}$ is the absorbance of the control and the A_{sample} is the absorbance of the test sample containing the mixture.

Analysis of Phenolic Content

The total phenolic content was estimated using Folin-Ciocalteu method. Fifty µl of sample solution was mixed with 500 µl of 10% Folin-Ciocalteu reagent (Folin:Methanol, 1:1, v/v). Fifty µl of 7.5% saturated was added and kept in the dark for 1h before taking the absorbance at 765 nm. The total phenolic content of the sample will be expressed as mg of gallic acid equivalents (GAEs) per gram of sample.

Determination of Cytotoxicity

The toxicity of mushroom extract was assessed using brine shrimp assay. The different concentrations (1, 10, 100, 1000 and 10000 µg/mL) of extract were prepared and forty (40) nauplii were exposed in each replicate. Triplicate was done each concentration. After 6 hours of exposure, the number of dead nuplii was recorded and the percentage mortality was computed. LC50 value was computed in order to determine the cytotoxicity level based on the established Clarkson toxicity index.

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) was used to compare the treatment means at 5% level of significance. In cytotoxicity study, LC50-values at 95% of confidence intervals were determined.

RESULTS AND DISCUSSION

Mycochemical Constituents of *P. grammocephalus*

Mycochemicals are abundant in mushrooms, enabling them to perform various metabolic processes. In this study, the mycochemical screening was carried out to detect the secondary metabolites present in the fruiting bodies of mushroom using the protocol of Guevara *et al.* **[20]** and the results are presented in Table 1. Apparently, the fruiting bodies of *P. grammocephalus* contain essential oils, phenols, triterpenes, steroids, fatty acids, sugars, anthraquinones, coumarins, anthrones, tannins, flavonoids, and alkaloids. However, terpenoids, cardiac glycosides and saponins were not detected in the sample.

Table 1. Mycochemical compositions of *P. grammocephalus* fruiting bodies

Mycochemical	Results
Essential oils	Present
Phenols	Present
Triterpenes	Present
Steroids	Present
Fatty acids	Present
Sugars	Present
Anthraquinones	Present
Coumarins	Present
Anthrones	Present
Tannins	Present
Flavonoids	Present
Alkaloids	Present
Terpenoids	Not detected
Cardiac glycosides	Not detected
Saponins	Not detected

Essential oils, also known as volatile or ethereal oils, are aromatic oily liquids known for their antiseptic (bactericidal, virucidal and fungicidal), medicinal properties and fragrance. Because of this, essential oils are used in embalment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies **[12]**. Phenolic fractions and antioxidant properties of mushrooms are related. Aside from antioxidant properties, phenolics exhibit antimutagenic, antiviral, antibacterial, algicidal, antifungal,insecticidal, estrogenic and keratolytic activities that may protect the organism from competing ones in their biological environment **[13, 21].** Triterpenes, on the other hand, directly suppress growth and invasive behavior of cancer cells **[22, 23, 24, 25]**. Dudhgaonkar **[22]** reported the anti-inflammatory activities of isolated triterpenes from *Ganoderma lucidum*.

Fatty acids, the straight-chain monounsaturated and polyunsaturated and

branched chain building blocks of dietary fats and oils, have potential in the regulation of lipid metabolism at different levels **[26, 27]**. In the study of Yilmaz *et al.* **[26]**, it was revealed that linoleic acid, palmitic acid, oleic acid, stearic acid andarachidic acids are present in the species *Agaricus bisporus*, *Agaricus campestris*, *Coprinus comatus*, *Boletus edulis*, *Pleurotus ostreatus*, *Oudemansiella radicatam* and *Armilleria mellea*. The high content of polyunsaturated fatty acids makes mushrooms recommended in the diets of individuals with high blood cholesterol **[28]**. Barros *et al.* **[29]** revealed that mannitol and trehalose are present in *Agaricus arvensis*, *Leucopaxillus giganteus*, *Lactarius deliciosus*, *Sarcodon imbricatus*, and *Tricholoma portentosum*.

On the other hand, anthraquinones are aromatic organic compounds that are highly crystalline solid and are ingredients to many dyes. Medically, anthraquinone derivatives can be used as laxatives, antimalarials, and antineoplastics. Coumarins are fragrant organic chemical compounds used in the pharmaceutical industry for synthetic anticoagulants and fibrinolytics. Coumarins are also used as edema modifier and stimulator to macrophages in degrading extracellular albumin. Meanwhile, anthrone is a tricyclic aromatic ketone used in pharmacy as laxative. Anthrones stimulate the motion of the colon and reduce water reabsorption. *Pleurotus florida* has been reported to contain anthrones **[30]**.

Tannins have been used against heart diseases because of its ability in scavenging free radicals **[31, 32]**. Flavonoids possess antioxidant, anti-inflammatory, antiallergic, antiviral, as well as anticarcinogenic activities. Alkaloids were found to exist in *P. grammocephalus*, same is true with *Panaeoulus cyanescens* **[31]** and *Panaeolus pumonarius* **[33]**. Alkaloids are toxic to foreign cells, hence making it an interest for the prevention and reduction of cancer.

Antioxidant Activity of *P. grammocephalus*

Antioxidants protect the human body against oxidative damage caused by free radicals **[6, 34]**. Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress **[28]**. A stable free radical, 2,2'-diphenyl-1,1-picrylhydrazyl (DPPH), is often utilized in the analysis if a compound or extract has antioxidant properties. It has a characteristic absorption at 517 nm which decreases based on the ability of the antioxidants to provide an electron to the DPPH radical. In this study, the radical scavenging activity of *P. grammocephalus* is evaluated and results are

shown in Table 2. The radical scavenging activity of *P. grammocephalus* is found to be at 26.37%. This clearly indicates that *P. grammocephalus* has promising potential in the pharmaceutical industry because antioxidants perform important functions in preventing diseases related with free radicals. Phenolic compounds possess scavenging ability because of their hydroxyl groups, making them one of the most significant bioactive compounds with antioxidant activity. Phenols were found to have activities against heart ailments and cancer, and the ability as anti-inflammatory. In this study, the total phenolic content of *P. grammocephalus* was also analyzed (Table 2). Based on the result, the fruiting bodies of *P. grammocaphalus* contain phenolics with a mean 38.58 mg GAE / g sample. This strongly suggests that the fruiting bodies of *P. grammocephalus* could be source of active phenolics as potent antioxidant.

Table 2. Radical scavenging activity and total phenolic content of *P. grammocephalus*

Extract	Radical Scavenging Activity (%)	Total Phenolic Content (mg GAE/g sample)
P. grammocephalus	**26.37**	**38.58**
Cathechin (control)	**81.35**	**-**

Cytotoxic Effect of *P. grammocephalus*

In order to establish the cytotoxic effect, if any, of the extract of *P. grammocephalus* fruiting bodies. The brine shrimp lethality assay was carried out and the LC50 value was computed. The LC50 value of the extract was 73.78 µg/ml. This value revealed that the the extract of fruiting bodies of *P. grammocephalus* is highly toxic based on the toxicity levels established by Clarkson et al. **[35]**. This important result implies that this mushroom has great potential as source of toxic compounds that can be used in further evaluation of other biological activities such as anti-inflammatory, antimicrobial, and anticancer, which we need to investigate.

CONCLUSION

It can be concluded therefore that the fruiting bodies of *P. grammocephalus* are rich in bioactive secondary metabolites, and its ethanolic extract exhibits antioxidant properties and cytotoxic effect.

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