Phytochemical Characteristics Evaluation of *Pleurotus species* Cultivated on Agricultural Wastes in Chiro, Ethiopia

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**ABSTRACT**

**Background:** Mushrooms are a nutritious food source, being rich in protein, vitamins and minerals. They are also contains substances that enhance the immune system, fight infectious disease. Mushrooms can be cultivated on a variety of substrates, including agricultural and agro-industrial waste materials. The current study was aimed to evaluate the phytochemical characteristics of *Pleurotus species* cultivated on different agricultural wastes.

**Methods:** Mushrooms *Pleurotus ostreatus* and *Pleurotus florida* were cultivated on different agricultural wastes for the screening of phytochemical characteristics. Qualitative analyses of the phytochemicals were evaluated in methanolic, ethanolic and aqueous extracts of both *Pleurotus* spp. Total phenolic and total flavonoid contents of the extracts were determined by using Folin-Ciocalteu method and Spectrophotometric method with aluminum chloride.

**Result:** Qualitative analyses revealed the phytochemicals alkaloids, saponins, flavonoids and tannins were present in methanolic, ethanolic and aqueous extracts of both *Pleurotus* spp. while anthraquinones and Phlobatannins were absent in aqueous extracts. The highest concentration of phenols and flavonoids were recorded in methanolic extracts of *P. ostreatus* and *P. florida* (48.17 mg GAE/g of extract and 56.57 mg of RUE/g of extract and 46.73 mg GAE/g of extract and 55.58 mg of RUE/g of extract respectively). The results supported the methanolic extracts of *P. ostreatus* and *P. florida* might indeed be potential sources of phytochemicals.

**Key words:** Agricultural wastes, Flavonoids, Phenols, Phytochemical, *Pleurotus* spp.

**INTRODUCTION**

Edible mushrooms have gained worldwide recognition and increasing popularity owing to their nutritional and medicinal values since Greek and Roman antiquity. (Pushpa and Purushothama, 2010; Gan et al. 2013). People usually use fruit bodies and sclerotia of edible mushrooms as major food condiments that are served at their important family meals. Edible macro fungi are usually collected from the wild because farms growing them are very few (Jonathan et al. 2012). Mushrooms possess high contents of qualitative protein, crude fibre, minerals and vitamins. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. They produce a wide range of secondary metabolites with high therapeutic value. Health promoting properties, e.g. antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects, have been reported for some species of mushrooms. Both fruiting bodies and the mycelium contain compounds with wide ranging antioxidant and antimicrobial activities. (Marijana et al. 2012). In recent decades, various extracts of mushrooms and plants have been of great interest as sources of natural products (Aziz et al. 2007).

The oyster mushroom *Pleurotus* spp. is widely cultivated on a wide range of substrates which are composed of lignin and cellulose. Cultivation of *Pleurotus* spp. supports a broad range of temperatures (15-30°C) on different range of substrates like agro waste residues, weeds and wastes after the production of food, feed, vitamins, enzymes and a number of pharmaceuticals in addition to their waste degradation and detoxification properties (Gregori et al. 2007; Jonathan, 2012). The bioactive compounds present in *Pleurotus* spp. makes it a medicinally important mushroom (Gregori et al. 2007). There is an evidence that substrate composition influences the chemical composition and antimicrobial activity of mushroom extracted for aqueous extracts (Kérley et al., 2011).

In recent years, high scale usage of synthetic antibiotic leads the emergence of multi drug resistance pathogens, is now posing a threat to the world. The resistance among various microbial species (infectious agents) to different antimicrobial drugs has emerged as a cause of public health threat all over the world at a terrifying rate. Due to the pacing advent of new resistance mechanisms and decrease in efficiency of treating common infectious diseases, it results in failure of microbial response to standard treatment,
leading to prolonged illness, higher expenditures for health care and an immense risk of death (Jyoti et al. 2014). Therefore, a search for natural plant based antimicrobial agents is in need. This development is the consequence of the limited effectiveness of synthetic products to fight against newer and drug resistant bacteria. For this purpose, the antimicrobial properties of many natural compounds from a wide variety of plant species have been assessed (Karuppusamy, 2009).

Phenolic and flavonoid compounds have attracted much interest recently because in vitro and in vivo studies suggest that they have a variety of beneficial biological properties which may play an important role in the maintenance of human health. Their significance in the human diet and their antimicrobial activity have been recently established. Antioxidant and antimicrobial activities of Pleurotus species were reported and correlated to the phenols and flavonoids contents (Barros et al. 2007). In Ethiopia, no studies have been conducted for Evaluation of Phytochemical Characteristics of Pleurotus spp. cultivated on different Agricultural wastes and little is known of the biology and potential phytochemical sources of Pleurotus species grown on different agricultural waste in spite of its nutritional importance. By considering this problem, attention needs to be given to investigate its phytochemical characteristics which could serve as an input for efficiently manage microbial disease. Therefore, this study was undertaken with the objectives of Evaluation of Phytochemical Characteristics of Pleurotus species cultivated on different agricultural wastes.

**Materials and Methods**

**Experimental site**

All experiments were carried at the Mycological Research Laboratory and Mushroom greenhouse of the Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences at Addis Ababa University, Ethiopia in 2017.

**Spawn collection**

The mother spawn of Pleurotus florida and Pleurotus ostreatus were obtained from Addis Ababa University.

**Spawn preparation**

Spawn were prepared by using method of Bano and Shrivastava (1962) with slight modifications. One kg of wheat grain was cooked for 40 min after that washed in tap-water. Grain was drained and supplemented with 2 g lime and 8 g gypsum and mixed manually. Then grain was filled in polypropylene (PP) bags of 1 Kg capacity and sterilized in autoclave at 121°C for 15 min. After cooling, PP bag was inoculated with freshly prepared mycelium (previously prepared PDA plate) and incubated at 25°C for two weeks in an incubator.

**Preparation of mushroom extract**

The present study was carried out to know the phytochemical potentiality Pleurotus spp. (P. ostreatus and P. florida) mushrooms cultivated on different agricultural wastes namely Coffee straw (CS), Pea straw (PS), Sorghum Grain Residue (SGR) and Wheat Grain (WG). Freshly harvested fruiting bodies from P. ostreatus and P. florida were shade dried and finely powdered. Twenty grams of the powder were extracted with 200 ml of 95% solvent methanol, ethanol and aqueous separately using soxhlet apparatus. The remaining extract was filtered and evaporated by vacuum distillation; the filtrate thus obtained was used as mushroom extract (Jayakumar et al. 2009). The extraction was twice repeated.

**Preliminary phytochemical characteristics**

Preliminary phytochemicals were qualitatively analyzed by using methods of Trease and Evans (1994) and Harborne (1973) for alkaloids, tannins, saponins, Anthraquinones, steroids, phlobotannins, phenols and flavonoids.

**Determination of total phenolics**

The total phenolic content was determined by the Spectrophotometric method (Kim et al. 2003). In brief, a 1 ml of sample (1 mg/ml) of methanolic, ethanolic and aqueous extract was mixed with 1 ml of Folin-Cioacatel’s phenol reagent. After 5 min, 10 ml of a 7% Na₂CO₃ solution was added to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 725 nm. The Total Phenolic Content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The Total Phenolic Content was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample. Total content of phenolic in the sample extracts in gallic acid equivalent (GAE) was calculated by the following formula (Imran et al. 2011):

\[
T = \frac{c \times V}{m}
\]

Where

- \(T\) is the total content of phenolic compounds, mg/g fresh material, in GAE;
- \(c\) the concentration of gallic acid established from the calibration curve, mg/l;
- \(V\) the volume of extract, L;
- \(m\) is the weight of extract, g.

**Determination of total flavonoids**

Total flavonoid content was determined using the Dowd method as adapted by Arvoulet-Grand et al. (1994). Briefly, 1 ml of 2% aluminum trichloride (AlCl₃) in methanol was mixed with the same volume of the methanolic, ethanolic and aqueous extract (2,000 μg). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 ml extract solution with 1 ml methanol without AlCl₃. The standard curve for total flavonoids was made using rutin standard solution (0 to 100 mg/l) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of rutin equivalents per g of dried fraction.
Data entry and analysis
All data were analyzed and expressed as mean ± standard deviations of three separate determinations (n=3). The statistical analysis was carried out by using SAS for Windows, version 9.1. One-way analysis of variance (ANOVA) and LSD comparisons were carried out to detect significant difference (p < 0.05) between the mean values that had more than two groups.

RESULTS AND DISCUSSION
Preliminary phytochemical characteristics
Methanolic, Ethanollic and Aqueous extracts of Pleurotus species revealed the presence of secondary metabolites like alkaloids, steroids, tannins, saponins, phlobatannins and Anthraquinones. The methanolic extracts have shown the presence of most of secondary metabolites and these were confirmed by methanolic extraction. However, some secondary metabolites extracted only with aqueous solvent and some with ethanol solvent.

Methanolic extracts have shown a greater number of phytoconstituents (37 positive result) and then ethanollic extracts (33 positive result) than aqueous extracts (24 positive result) which is to be high lightened (Tables 1, 2 and 3) because successive isolation of phytoconstituents from mushroom species is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily Methanol as the solvent (Padmavathy and Mekala, 2013) thus is a positive consideration that phytochemical screening, has shown methanolic extract is found to be more effective than ethanolic and aqueous extract.

Among two selected mushroom species methanolic, ethanolic and aqueous extracts of mushroom species has shown the presence of phytoconstituents like alkaloids, tannins, saponins, phlobatannins and Anthraquinones. But Phlobatannins and Anthraquinones are not detected in aqueous extracts of both P. ostreatus and P. florida by using different agricultural wastes as indicated in Table 3. In addition, phytochemicals like phlobatannins and steroids are completely not detected in methanolic and aqueous extracts of P. florida respectively by using different agricultural wastes as shown in Table 1 and 3 as indicated by the negative sign.

Tannins found present in all methanolic, ethanolic and aqueous extractions of mushroom species. Tannins show anti-carcinogenic, anti-mutagenic, anti-oxidative, anti-microbial properties, also exert other physiological effects such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level and modulate immunoresponses (Chung, 1998). Saponins found present in all but absent only in the pea straw ethanolic extraction of P. florida. Presence of saponins in mushroom extracts of all species except pea straw substrate of P. florida indicates anti-diarrheal, anti-helminthic, anticancer activities of these mushrooms (Padmavathy and Mekala, 2013). Phlobatannins have shown positive test with methanolic extracts of pea straw and wheat grain of P. ostreatus and ethanolic extraction of sorghum grain residue substrate of P. florida. Whereas, methanolic extracts of P. florida ethanolic and aqueous extractions of both mushroom species shown

| Phytochemicals | Test performed | P. ostreatus | P. florida |
|----------------|----------------|-------------|------------|
| Methanolic extracts | | CS | SGR | PS | WG | CS | SGR | PS | WG |
| Alkaloids | Mayer’s Test | + | + | + | + | + | + | + | + |
| Tannins | Lead acetate Test | + | + | + | + | + | + | + | + |
| Saponins | Frothing Test | + | + | + | + | + | + | + | + |
| Steroids | Ring Test | + | + | + | + | + | + | + | + |
| Anthraquinones | Borntrager’s reaction | + | + | + | - | + | - | - | + |
| Phlobatannins | Boiling Test | - | - | + | - | - | - | - | - |

+ indicate presence - indicate absence.

| Phytochemicals | Test performed | P. ostreatus | P. florida |
|----------------|----------------|-------------|------------|
| Ethanollic Extracts | | CS | SGR | PS | WG | CS | SGR | PS | WG |
| Alkaloids | Mayer’s Test | + | + | + | + | + | + | + | + |
| Tannins | Lead acetate Test | + | + | + | + | + | + | + | + |
| Saponins | Frothing Test | + | + | + | + | + | - | - | - |
| Steroids | Ring Test | + | + | + | - | + | - | - | - |
| Anthraquinones | Borntrager’s reaction | - | - | + | + | + | + | + | + |
| Phlobatannins | Boiling (HCl) Test | - | - | - | - | - | - | - | - |

+ indicate presence - indicate absence.
Phytochemicals detected in aqueous extracts of mushroom species.

| Phytochemicals | Test performed          | Aqueous Extracts | P. ostreatus | P. florida |
|----------------|-------------------------|------------------|--------------|------------|
|                |                         | CS  | SGR | PS  | WG | CS  | SGR | PS  | WG |
| Alkaloids      | Mayer’s Test            | +   | +   | +   | +  | +   | +   | +   | +  |
| Tannins        | Lead acetate Test       | +   | +   | +   | +  | +   | +   | +   | +  |
| Saponins       | Frothing Test           | +   | +   | +   | +  | +   | +   | +   | +  |
| Steroids       | Ring Test               | +   | +   | +   | -  | -   | -   | -   | -  |
| Anthraquinones | Borntrager’s reaction   | -   | -   | -   | -  | -   | -   | -   | -  |
| Phlobatannins  | Boiling(HCl) Test       | -   | -   | -   | -  | -   | -   | -   | -  |

+ indicate presence   - indicate absence

Table 3: Phytochemicals detected in aqueous extracts of mushroom species.

The average yields of solid residue after extraction and evaporation from 10 g dried Mushroom species.

Table 4: The average yields of solid residue after extraction and evaporation from 10 g dried Mushroom species.

Table 5: Total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

Total phenolic and flavonoid content of mushroom species extracts

Methanolic, ethanolic and aqueous extracts were prepared to examine the total phenolic and flavonoid content. The yield of extract obtained from 10 g of dry Mushroom was measured for each extract (Table 4). The highest yield of solid residue was obtained using methanol as extraction solvents for both mushroom species. The lowest yield of solid residue was obtained in aqueous extracts of both mushroom species as shown in Table 4.

The total phenolic contents in the examined mushroom extracts using the Folin-Ciocalteu’s reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 7.026x - 0.0191$, $r^2 = 0.998$). The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract (Table 5). The total phenolic contents in the examined extracts ranged from 25.44 to 48.17 mg GAE/g. The highest concentration of phenols from P. ostreatus was measured in methanolic and ethanolic extracts. Similarly, the highest concentration of phenols from P. florida was measured in methanolic and ethanolic extracts. Aqueous extracts of P. ostreatus and ethanolic and aqueous extracts of P. florida contains considerably smaller concentration of phenols. The total phenolic content in mushroom extracts of the species depends on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these phenols in aqueous extracts.
compounds in the extracts obtained using polar solvents for the extraction (Mohsen and Ammar, 2008). Phenols acts as antioxidants also associated with the inhibition of atherosclerosis and cancer (Martínez et al. 2000).

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of mushrooms may contribute directly to their antioxidant action (Tosun et al. 2009). The extraction of phytochemical substances of different chemical structure was achieved using solvents of different polarity. Numerous investigations of qualitative composition of plant extracts revealed the presence of high concentrations of phenols in the extracts obtained using polar solvents (Čanadanović-Brunet et al., 2008). The extracts that have the highest concentration of phenols perform the highest antioxidant activity (Čanadanović-Brunet et al. 2008). Similar trends have been reported by Azieana et al. (2017), where maximum phenolic and flavonoid content seen for wild edible mushroom.

The concentration of flavonoids in various mushroom extracts of the species P. ostreatus and P. florida was determined using Spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of rutin equivalent (the standard curve equation: y = 17.231x – 0.0591, r² = 0.999), mg of RUE/g of extract (Table 6). The concentration of flavonoids in mushroom extracts from P. ostreatus ranged from 20.52 to 56.57 mg RUE/g. Similarly, the concentration of flavonoids in mushroom extracts from P. florida ranged from 16.42 to 55.58 mg RU/g. Methanolic and ethanolic extracts contains the highest flavonoid concentration and aqueous extract contains the lowest flavonoid concentration. The concentration of flavonoids in methanol extract of P. ostreatus was 56.57 mg RUE/g, which was very similar to the value of methanolic extract concentration of P. florida. The lowest flavonoid concentration was measured in aqueous extract of P. ostreatus and P. florida. The concentration of flavonoids in Mushroom extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005). Presence of flavonoids in mushroom species has revealed anti-inflammatory, antioxidant, anticancer, antibacterial and antiviral properties (Harborne and Williams, 2001). Similar studies revealed that the flavonoid content of medicinal mushroom species is natural sources of antioxidant substances of high importance. It is noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvents of high polarity; the methanolic extract manifested greater power of extraction for phenolic compounds from P. ostreatus and P. florida. The flavonoid in the presence of aluminum chloride have an intense yellow fluorescence when observed UV spectrophotometer. (Luis et al. 2009). The result obtained from the study is expected to show which Pleurotus species have high content of total phenols and flavonoids, which is in correlation with intense antioxidant activity of these extracts. Similar studies conducted by Harborne and Williams, 2001 revealed that the phytochemical constituents of medicinal plants and mushroom have significant vales for antioxidant properties and its phytochemical constituent may range from 15-75mg/g of extract). Similarly, Marijana et al. (2012) revealed that, high content of phenolic and flavonoid content was extracted from mushroom species by using different extraction solvents like acetone and methanol. Similar result was reported by Fai-Chu W et al. 2013).

**CONCLUSION**

Findings of qualitative analysis of phytochemicals of P. ostreatus and P. florida showed that the methanolic, ethanolic and aqueous extracts contain alkaloids, tannins, saponins, flavonoids, steroids and glycosides as reported by Okwulehie and Ogoke (2013). Phytochemicals present in methanolic and aqueous extracts of P. florida was supported by studies of Menaga et al. (2012). Methanolic and ethanolic extract of P. ostreatus and P. florida showed the highest phenolic and flavonoid concentration. P. ostreatus and P. florida can be regarded as promising candidates for natural sources of antioxidants with high value. The results of this study suggest that, the great value of the species P. ostreatus and P. florida for use in pharmacy and phytotherapy. Based on this information, it could be concluded that this mushroom species is natural sources of antioxidant substances of high importance. It is noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvents of high polarity; the methanolic extract manifested greater power of extraction for phenolic compounds from P. ostreatus and P. florida. The flavonoid in the presence of aluminum chloride have an intense yellow fluorescence when observed UV spectrophotometer. (Luis et al. 2009). The result obtained from the study is expected to show which Pleurotus species have a high content of phytochemicals. So, the Pleurotus species are employed as an alternative source of medicine to mitigate the diseases associated with microorganism. In addition, it helps to compare level of phytochemicals of the Pleurotus species. In general, the present finding encourages their use in human diets, which in turn might serve as potential source of phytochemicals.

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