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Research Article

UTILIZATION OF VEGETABLE WASTE FOR THE CULTIVATION OF *P. OSTREATUS* AND ITS INFLUENCE ON PHYTOCHEMICALS

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ABSTRACT
Pleurotus ostreatus, Oyster mushroom is one of the common edible mushroom and is the second largest to be cultivated. It belongs to the Class Basidiomycetes and Family Agaricaceae. It grows naturally in temperate and tropical forests on dead and decaying wooden logs and as well as on decaying organic matter. These mushrooms have been used for thousands of years as a culinary and medicinal ingredient. It is one of the most suitable fungal organism for producing protein rich food from various agro waste without composting. The present study proves the enrichment of nutrition in mushroom samples with utilisation of vegetable waste as substrate. The combination of vegetable waste and paddy straw gave better yield in comparison to paddy straw alone. The parameters tested were proximate analysis, qualitative estimation of phytochemicals followed by quantification of phenols and determination of minerals. The fruiting bodies cultivated on vegetable waste in combination of paddy straw accumulated higher concentration of total proteins (20.98mg/g), total carbohydrates (56.57mg/g) and total phenols (344µg /g) than the fruiting bodies cultivated on paddy straw alone which had high moisture content. Crude fat had no significant difference in both the mushroom samples. The present study revealed that the combination of vegetable waste with paddy straw was a better substrate in comparison to paddy straw alone. Also this combination is a promising substrate for cultivating oyster mushroom.

Keywords: *P. Ostreatus, Vegetable Waste, Qualitative and Quantitative Analysis*

INTRODUCTION

Mushrooms are considered as a functional food, which can provide health benefits beyond the traditional nutrients they contain. Nevertheless, until the last decade as compared with vegetables and medicinal mushroom species, knowledge of the composition and nutritional value of culinary mushrooms was limited. Culinary mushrooms have been perceived only as a delicacy and their consumption in many developed countries has been marginal and thus, of little interest to researches.

Among the abundant number of edible mushrooms *Pleurotus* genus is a prolific produced of novel mycochemicals. The origin of *Pleurotus* was first cultivated during the First World War in Germany as a subsistence measure for food storages and the first documentation of cultivation was done by Kaufer (1936). Nowadays, several species of *Pleurotus* are cultivated commercially because of their rich mineral contents and medicinal properties, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes and low demand on resources and technology (Yildiz *et al.*, 2002). And also the substrate used for the harvesting of the *Pleurotus* mushroom is valuable as a fertilizer and a soil conditioner for the growth of plants (Brenneman *et al*., 1994).

Additionally, fermented residues could be used as animal feed after mushroom cultivation (Soto-Cruz *et al*., 1994). Thus, the cultivation process of *Pleurotus* can solve one of the most important problems in soil waste disposal, economical gain and protect the environment. Many investigations from different regions of the world confirmed that the *Pleurotus* mushroom are high in nutrition and also contain various bioactive compounds including terpenoids, steroids, phenols, alkaloids, lectins and nucleotides, which have been isolated and identified from the fruit body, mycelium and culture broth of mushrooms are shown to have promising biological effects (Lindequist *et al.*, 2005). The present study aims at harvesting oyster mushrooms from combination of 50% Paddy straw and 50% vegetable waste and also paddy straw alone and analysing proximate composition, biomolecules, phytochemicals and mineral composition.

MATERIALS AND METHODS

Sample Collection: Fruiting bodies were harvested from combination of 50% Paddy straw and 50% vegetable waste comprising of carrot, onion, radish, cucumber and potato peels and from 100% paddy straw as substrate were collected. They were packed in perforated polythene bags and stored in refrigerator to keep fresh.

The mushrooms harvested from combination of paddy straw and vegetable waste were treated as test and that from paddy straw alone were treated as control.

Proximate Analysis

Moisture Content: 1g of both the control and test sample was weighed in a clean and dry china dish. These samples were dried at 110ºC in the hot air oven for 30 minutes. This was repeated till the constant weight was obtained. From the measured weights the moisture content was calculated (Sonali, 2012).

Total Ash Content: 1g of each sample was weighed in a clean and dry silica crucible and it was placed in muffle furnace for 30 minutes at 600ºC.

The crucible was left in the furnace till it cooled, weighed and then calculated (Oyetayo and Akindahunsi, 2004).

Total Crude Fat Content: The fat content was determined after extraction using the soxhlet apparatus. Ground sample (5g) was wrapped in a filter paper and placed into the soxhlet extractor. Petroleum ether was used as the solvent to extract fat and the process was continued till the completion of five cycles. Following the recovery the fat content was determined from the solvent extracted (Anakalo *et al*., 2008).

Quantitative Estimation of Sample Extract:

Total Proteins

Preparation of Sample: A 5% buffer extract of both the control and test mushroom samples were taken and was homogenized by adding 5mL of Tris HCl buffer of pH = 7.2. This mixture was centrifuged at 12,000 rpm for 10 minutes and the supernatant was used for estimation.

Estimation by Lowry's Method: The total protein content of both the sample extract was determined by following a slightly modified method of Lowry *et al.,* (1951). It was expressed as mg/gm equivalents of BSA. The number of trials was subjected to mean and standard deviation calculation.

Total Carbohydrates

Preparation of Sample: A 5% extract was prepared in 2.5N HCl by hydrolysis in boiling water bath for 3hrs and cooled to room temperature. The contents were neutralized with $NaCO_3$ until effervescence ceased.

Then the volume was made up with distilled water and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and aliquots were used for estimation.

Estimation by Anthrone Method: Total carbohydrate was estimated by anthrone method (Yemm and Willis, 1954). It was expressed as mg/gm equivalents of glucose. The numbers of trials were subjected to mean and standard deviation calculation.

Total Phenols

Preparation of Sample: A 5% extract was prepared in methanol. The extract was filtered using whattman filter paper and the filtrate was subjected to evaporation by keeping it on hot plate at 50ºC till the volume reduced to half of the original volume. The obtained volume was made up to 10mL using water: methanol mixture (1:1 ratio). Aliquots of this extract were used for the estimation of total phenols.

Estimation of Total Phenols: The total phenolic content of the 5% extract was determined by using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). It was expressed as gallic acid equivalents µg/g.

Qualitative Analysis of Phytochemicals

The fresh mushroom samples were subjected to various phytochemical screening using 5% methanol extract of both control and test sample.

Test for Carbohydrates

Fehling's Test: The sample extract was treated with 1 ml of Fehling's A and B and heated in a boiling water bath for 5-10min. Appearance of reddish orange precipitate showed the presence of carbohydrates (AOAC, 1995; Chaturvedi *et al*., 2013).

Test for Proteins

Biuret Test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added to sample extract. Appearance of pink or purple color indicated the presence of proteins and free amino acids (Sofowora, 1993; Trease *et al*., 1989).

Test for Fixed Oils and Fats: Small quantity of the various extracts was separately pressed between two filter papers. Appearance of oil stain on the paper indicated the presence of fixed oil (Sofowora, 1993; Trease *et al*., 1989).

Test for Alkaloids: A small portion of the sample extract was stirred separately with a fewdrops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with various alkaloidal reagents such as Dragondroff's reagent and Hager's reagent (Neelapu *et al*., 2011).

Test for Tannins and Phenolic Compounds: Small quantity of sample extract was taken separately in water and tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%). Appearance of violet color showed presence of tannins and phenolics (Odebiyi and Sofowora, 1978; Sofowora, 1982).

Test for Flavonoids: With aqueous solution of sodium hydroxide blue to violet color indicated presence of anthrocyanins, yellow color indicated flavones, yellow to orange indicated flavonones (Harborne and Williams, 1971; Harborne, 1973; Harborne, 1998).

Test for Terpenoids: To the 5mL of sample extract 2mL of chloroform and 3mL of conc. H_2SO_4 was added and checked for the reddish brown coloration (Sofowora, 1993; Trease *et al*., 1989).

Test for Steroids: Libermann – Burchard reaction: 2 ml sample extract was mixed with chloroform. To this 1-2 ml acetic acid and 2 drops concentrated sulphuric acid were added from the side of test tube. First red, then blue and finally green colour appeared (Sofowora, 1982).

Test for Phytosterol: The sample extract was dissolved in few drops of dry acetic acid; 3ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green color showed the presence of phytosterol (Harborne and Williams, 1971; Harborne, 1973; Harborne, 1998).

Test for Cardiac Glycoside: Keller-Killani Test- To 2 ml of extract, glacial acetic acid, one drop of 5 % ferric chloride and concentrated sulphuric acid were added. Appearance of reddish brown color at the junction of the two liquid layers indicated the presence of cardiac glycosides (Sofowora, 1993; Trease *et al*., 1989).

Test for Anthraquinone Glycosides: Borntrager's Test – To 3 ml extract, dilute sulphuric acid was added, boiled and filtered. To the cold filtrate equal volume benzene or chloroform was added. The organic layer was separated and ammonia was added. Ammonical layer turned pink or red (Sofowora, 1993; Trease *et al.*, 1989).

Test for Saponin Glycosides: Foam test – The extract and acacia powder were mixed vigorously with water and observed for foam (Odebiyi and Sofowora, 1978; Ngbede *et al*., 2008).

Test for Coumarin Glycosides: Alcoholic extract when made alkaline, showed blue or green fluorescence (Mojab *et al*., 2003).

Estimation of Minerals

Sample Preparation: Mineral assays were made after dry ashing the mushroom samples which was treated with concentrated HCl and distilled water. The solution was kept on hot plate and evaporated to dryness. After cooling, the residue was dissolved in distilled water and filtered using whattman filter paper. The filtrate was made upto 50mL in volumetric flask using milli Q water and used for Na^+ and K^+ estimation.

***Estimation of Na and K by Flame Photometry*:** Prior to determination of Na and K, the appropriate stock solutions (1000 ppm) were prepared for both the minerals. From the stock solution, 20, 40, 60, 80 and 100ppm working standard solution was prepared using milli Q water.

To calibrate the instrument, milli Q water was used and then replaced the water with working standard solutions and the readings were noted. Water is run through the instrument again for 2 minutes and draw tube was placed into a beaker containing the sample solution to be estimated and the readings are noted down. The filter is changed simultaneously while performing the experiment. Finally, run water through the instrument until the flame appears free of color (Overman and Davis, 1947).

RESULTS AND DISCUSSION

Proximate Composition Analysis

Moisture Content: Mushroom being fleshy and succulent contains high content of moisture. It is observed that the control sample comprised of marginally higher moisture content (90%) when compared to that of test (85%) as shown in the Figure 1(a). Zahid *et al*., (2009) reported that moisture content was found in a range of 85.95-90.07%.

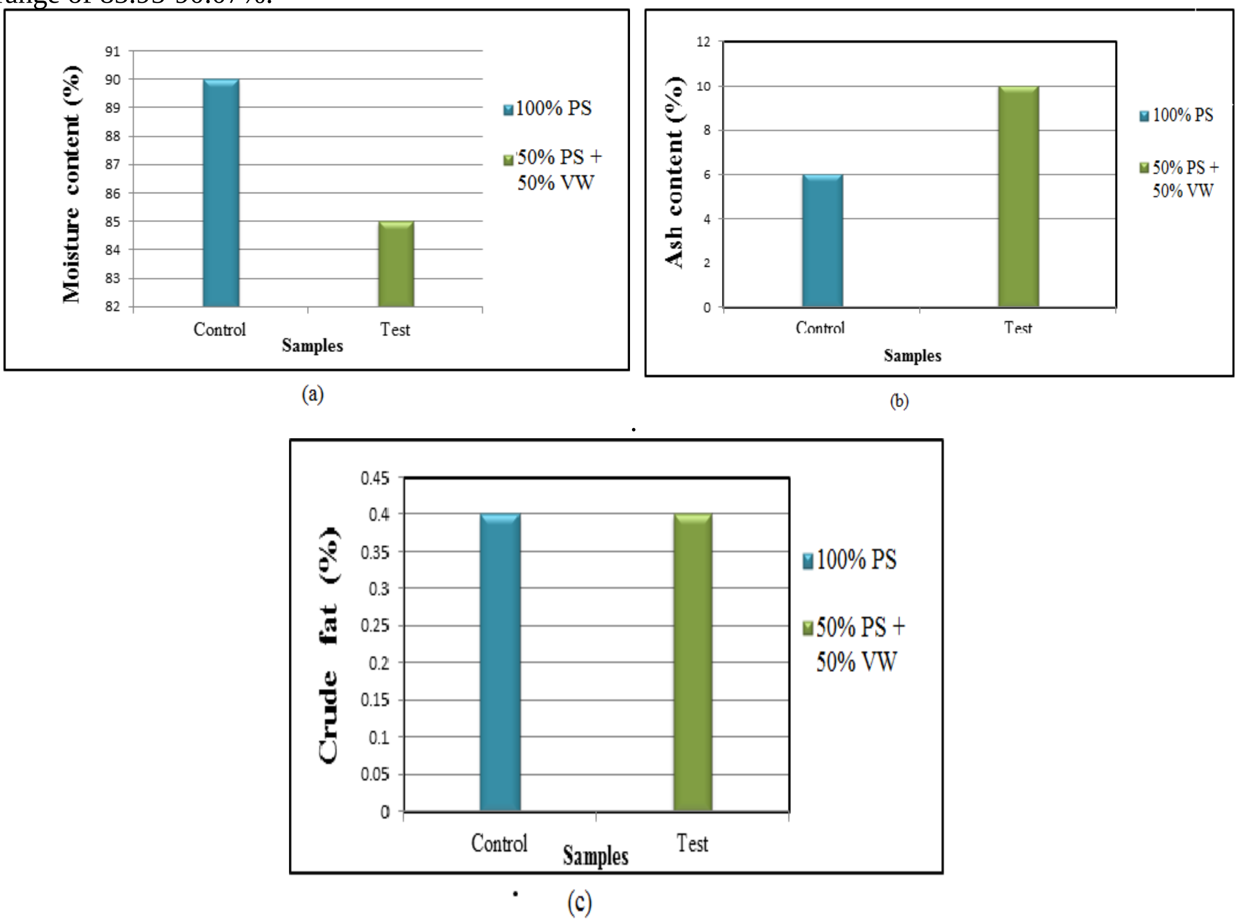

Figure 1: Effect of Substrate on (a) Moisture, (b) Ash and (c) Crude Fat Content

Ash Content: Ash content signifies the amount of dry matter constituents. It was found to be 4% higher in the test sample (10%) when compared to the control (6%) as depicted in the Figure 1(b). Similar studies conducted by Ahmed *et al*., (2009) showed that ash content was found to be 6.6%.

Crude Fat: Mushrooms are known for their nutraceutical value due to less amount of lipid or fat content when compared to other vegetables. The total crude fat was observed to be equal in both the samples with no significant difference (Figure 1(c)), indicating that the percentage of fat content was not altered by utilization of vegetable waste as substrate. 3.07±0.061 % of crude fat was seen in *P. osteratus* (Ashraf *et al*., 2013).

Quantitative Estimation of Sample Extract:

Estimation of Total Proteins: Oyster mushrooms are known for their richness in protein content. The test sample consisted of higher amount of proteins when compared to that of the control as shown in the Fig 2(a). This may be due to the greater mobilization of nitrogen from the nitrogen rich vegetable waste. The

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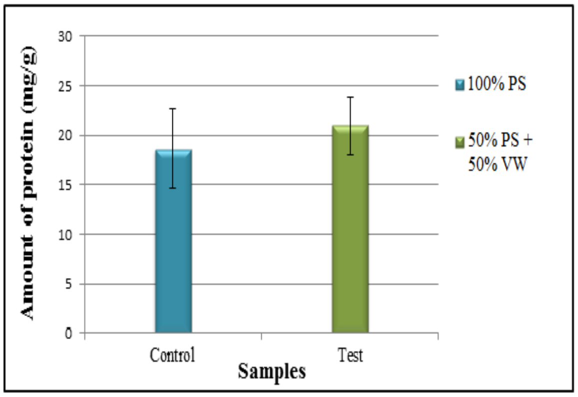
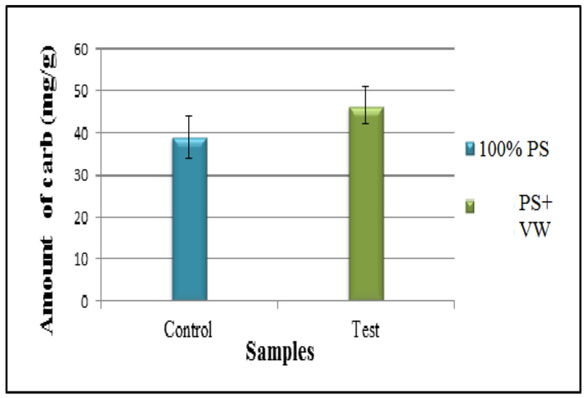
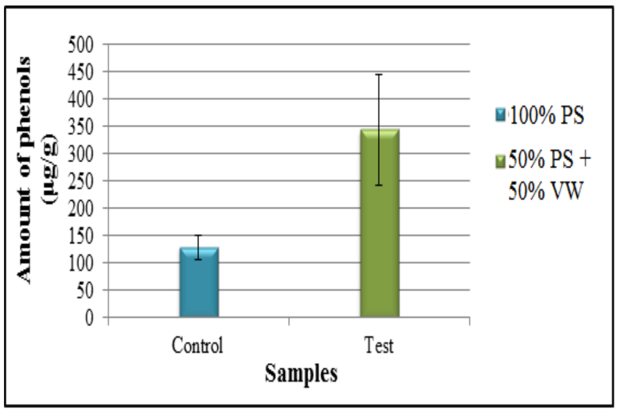
protein content determined in this study was found to be in accordance to the previous studies conducted (Kadam *et al*., 2008).



(a)



(b)



(c)

Figure 2: Effect of Substrate on (a) Total Protein, (b) Carbohydrate and (c) Phenolic Content

The experiment was performed in triplicate and results are presented as mean ±SD.

Estimation of Total Carbohydrates: Carbohydrates are the most important nutritional factor which is considered by many diets conscious consumers. The total carbohydrates were quantified to be 39.12mg/g of control sample and 56.57mg/g of test sample (Figure 2(b)). The results found in this section are approximately similar with Nasir *et al*., (2012). Table 1 depicts the results of proximate analysis.

Estimation of Total Phenols: The results of the total phenols estimation indicated a demarked increase in the quantity of total phenols in the test sample. This could prove the test sample to be a better antioxidant attaining maximum radical scavenging activity. The test sample comprised of 344µg/g of sample whereas the control sample contained only 128.87µg/g of sample as depicted in Figure 2(c). It was observed that the values obtained exceeded those that were reported earlier by Kortel *et al.*, (2014).

Table 1: Effect of Substrate on the Proximate Constituents of *P. Ostreatus*

	Moisture Content (%)	Ash Content (%)	Crude Fat (%)	Total proteins (mg/g of sample)	Total Carbohydrates (mg/g of Sample)
Control	90	6	0.4	18.63±4.06	32.13±5.17
Test	85	10	0.4	20.98±2.90	37.98±4.21

The experiment was performed in triplicate and results are presented as mean ±SD.

Qualitative Analysis

In the present study, the preliminary phytochemical analysis was carried out as depicted in Table 2. The result revealed the presence of carbohydrates, proteins, fixed oils and fats, alkaloids, terpenoids and glycosides such as cardiac glycoside in the methanolic extract of both the samples. Saponin glycosides were observed in the case of aqueous extract.

Table 2: Composition of Bioactive Compounds Extracted from *P. Ostreatus*

Bioactive Compounds	Control	Test
Carbohydrate	+	+
Proteins	+	+
Fixed oils and fats	+	+
Alkaloids	+	+
Tannins and phenolics	-	-
Flavonoids	-	-
Terpenoids	+	+
Steroids	-	-
Carotenoids	-	-
Phytosterols	-	-
Cardiac glycosides	+	+
Anthraquinone glycosides	-	-
Coumarin glycosides	-	-
Saponin glycosides	+	+

+ indicates presence
– indicates absence

Estimation of Mineral Ions: The mineral content of mushroom reveals the growth condition of mushroom. The flame photometric analysis showed that the sodium and potassium content was higher in the control sample when compared to the test sample (Figure 3: a & b). The control sample consisted of 18.55 ppm of sodium and 70.92 ppm of potassium ion contradictory to 7.18 ppm of sodium and 60.6 ppm of potassium ions in test sample.

The reduction of the ions in the test sample may be due to the utilization of these ions for the faster growth of fruiting bodies. In case of mineral estimation our study showed contrary results from that of Kortel and Wiafe-Kwagyan, (2015). This could be due to the variation in the substrate treatment and changes in the environmental conditions during the cultivation period.

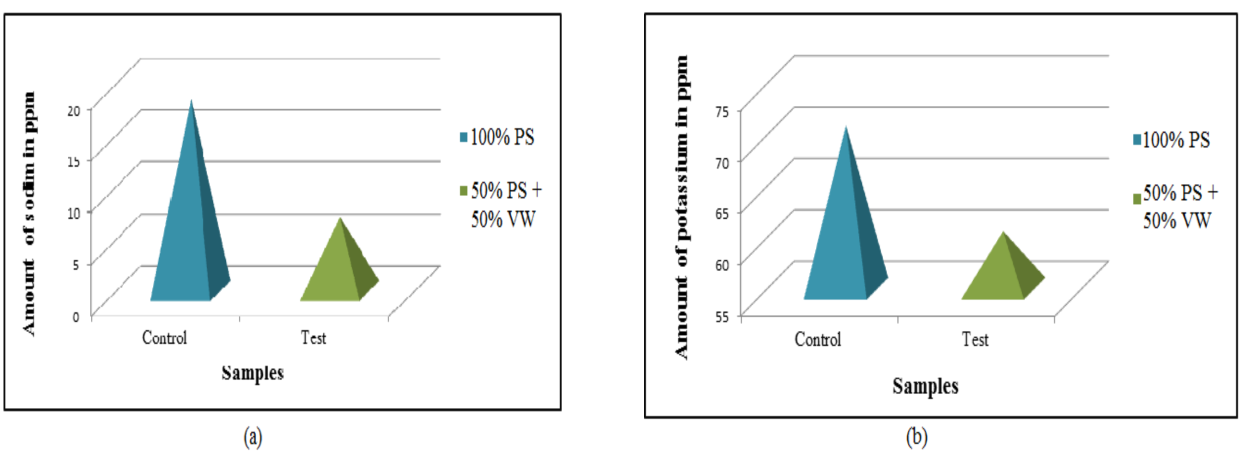


Figure 3: Effect of Substrate on Mineral Ion Content (a) Sodium Ion (b) Potassium Ion

Conclusion

Mushrooms are considered to be highly nutraceutical. They contain ample amounts of proteins, carbohydrates and are low in fat. Along with nutritive values they are also considered for their medicinal properties. The present study focuses on enhancement of the nutritive quality of these mushrooms by utilizing the combination of vegetable waste and agro waste as substrate. This provides prospects of converting residues into protein rich biomass.

For the study the fruiting bodies of mushroom cultivated on paddy straw and combination of paddy straw with vegetable waste were subjected to chemical analysis. The proximate components were analysed which included the total moisture content, ash content, crude fat content, total proteins and total carbohydrates.

Further on, the mushrooms were screened for different bioactive compounds. Total phenols and minerals such as sodium and potassium were quantified. The results showed that the mushrooms cultivated on 50% paddy straw in combination with 50% vegetable waste as substrate had enhanced nutritive value in comparison to the ones cultivated on paddy straw alone.

Hence, combination of vegetable waste and agricultural waste is a promising substrate for mushroom cultivation. Cultivation of these nutritionally rich mushrooms is also helpful to provide a mean to address the diet requirements of an individual. The catch lies in the cost effective production of these mushroom. It is a hassle free procedure to cultivate them at home environment or at the level of small scale industry with the added advantage of utilization of vegetable waste; we can bring out the “Best out of waste”.

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REFERENCES

Ahmed SA, Kadam JA, Mane VP, Patil SS and Baig MMV (2009). Biological efficiency and nutritional contents of *Pleurotus florida* (mont.) singer cultivated on different agro-wastes. *Nature and Science* **7** 44-48.

Anakalo KG, Shitandi AA, Mahungu MS, Khare KB and Sharma HK (2008). Nutritional composition of *Pleurotus sajor-caju* grown on water hyacinth, wheat straw and corncob substrates. *Research Journal of Agriculture and Biological Sciences* **4** 321-326.

AOAC (1995). *Official Methods of Analysis of the Association of Official Analytical Chemistry*. (AOAC International, Washington, USA) 1141- 7.

Ashraf J, Ali MA, Ahmad W, Ayyub CM and Shafi J (2013). Effect of different substrate supplements on oyster mushroom (*Pleurotus* sp.) production. *Food Science and Technology* **1**(3) 44-51.

Brenneman JA and Guttman MC (1994). The edibility and cultivation of the oyster mushroom. *American Biology Teacher* **56** 291-3.

Chaturvedi N, Sharma P and Agarwal H (2013). Comparative nutritional and phytochemical analysis of spinach cultivars: *B. alba* and *S. oleracea*. *International Journal of Pharma and Bio Sciences* **4** 674-679.

Harborne JB (1973). *Phytochemical Methods*, (UK, London: Chapman and Hall, Ltd.) 49-188.

Harborne JB (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, (UK, London: Chapman and Hall) **4** 302.

Harborne JB and Williams CA (1971). Leaf survey of flavonoids and simple phenols in the genus Rhododendron, *Phytochemistry* **9** 875-879.

Kadam RM, Patil SS and Jadhav BS (2008). Production of protein by fungi, *Pleurotus* species from different agricultural wastes. *International Journal of Plant Protection* **1** 45-47.

Kaufer F (1936). The biology of *Pleurotus corticatus* Fries. *Minnesota Agricultural Experiment Station Bulletin* **114**.

Khan NA, Khaliq N, Haq IU, Javed N and Gondal AS (2012). Impact of carbohydrates and mineral contents of different indigenous strains of oyster mushroom (jacq.fr.) cultivated on different agricultural wastes. *ESci International Journal of Phytopathology* **1** 56-61.

Kortel NK and Wiafe- Kwagyan M (2015). Comparative appraisal of the total phenolic content, flavonoids, free radical scavenging activity and nutritional qualities of *P. ostreatus* (EM-1) and *P. eous*(P-31) cultivated on rice straw in Ghana. *Journal of Advances in Biology and Biotechnology*. **3** 153-164.

Kortel NK, Dzogbefia VP and Obodai M (2014). Assessing the Effect of Composting Cassava Peel Based Substrates on the Yield, Nutritional Quality, and Physical Characteristics of Pleurotus ostreatus (Jacq.ex Fr.) Kummer. *Biotechnology Research International* **2014** Article ID 571520, 9 doi:10.1155/2014/571520.

Lindequist U, Niedermeyer THJ and Julich WD (2005). The pharmacological potentials of mushrooms. *Evidence-Based Complementary and Alternative Medicine* **2** 285-299.

Lowry OH (1951). Protein measurement with folin-phenol reagent. *The Journal of Biological Chemistry* **193** 265-275.

Mojab F, Kamalinejad M, Ghaderi N and Vahidipour H (2003). Phytochemical screening of Some Iranian plants. *Iranian Journal of Pharmaceutical Research* **3** 77-82.

Neelapu N, Naikwadi GD, Muvvala S and Jadhav KV (2011). A preliminary phytochemical screening of the leaves of *Solanum xanthocarpum*. *International Journal of Research in Ayurveda and Pharmacy* **2** 845-850.

Ngbede J, Yakubu RA and Nyam DA (2008). Phytochemical Screening for Active Compounds in Canarium scheinfurthii (Atile) leaves from Jos North, Plateau State, Nigeria. *Medwell Research Journal of Biological Science* **3**(9) 1076-1078.

Odebiyi A and Sofowora AE (1978). Phytochemical screening of Nigerian medicinal plants. *Lloydia* **41**(3) 234-246.

Overman RR and Davis AS (1947). The application of flame photometry to sodium and potassium determinations in biological fluids. *Journal of Biological Chemistry* **168** 641-649.

Oyetayo FL and Akindahunsi AA (2004). Nutrient distribution in wild and cultivated edible mushroom, *Pleurotus sajor-caju*. *Food, Agriculture & Environment* **2** 166-168.

Randive SD (2012). Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. *Advances in Applied Science Research* **3** 1938-1949

Singleton VL and Rossi JAJr (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Ecology and Viticulture* **16** 144-158.

Sofowora A (1993). *Medicinal Plants and Traditional Medicine in Africa*, (Nigeria, Ibadan, Spectrum Books Ltd) 289.

Sofowora A (1982). *Medicinal Plants and Traditional Medicinal in Africa*, (USA, New York: John Wiley and Sons).

Soto Cruz O, Saucedo-Castaneda G, Pablos Hach JL, Gutierrez- Rojas M and Favela-Tirres E (1999). Effects of substrate composition on the mycelia growth of *Pleurotus ostreatus* an analysis by mixture and response surface methodologies. *Process Biochemistry* **35** 127-33.

Trease GE and Evans WC (1989). *Pharamacognosy*, (W.B. Scandars Company Ltd., London, UK) **14** 269-300.

Yemm EW and Willis AJ (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemistry Journal* **57** 508-514.

Yildiz S, Yildiz ÜC, Gezer ED and Temiz A (2002). Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom. *Process Biochemistry* **38** 301–306.

Zahid MK, Barua S and Imamul Haque SM (2009). Proximate composition and mineral content of selected edible mushroom varieties of Bangladesh. *Bangladesh Journal of Nutrition* **22-23** 61-68.