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Phytochemicals and vitamin compositions of *Pleurotus pulmonarius* cultivated on barks of some indigenous fruit trees supplemented with agro-wastes

Okwulehie I. C. and Nosike E. N.

Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. Umuahia, Abia State, Nigeria

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ABSTRACT

The phytochemical compositions and vitamins contents of the fruit bodies of Pleurotus pulmonarius grown on different barks of fruit trees namely; Treculia africana (TA), Mangifera indica (MI), Dacryodes edulis (DE), Pentaclathra macrophylla (PM) and Elaeise guineensis (EG), supplemented with agro-wastes (Maize cobs, Fluted pumpkin wastes and Yam peels) were determined. Data obtained from these were subjected to ANOVA in a CRD and replicated 3 times with different levels of supplement per replicate. There was significant differences ($p<0.05$) within vitamin compositions of the fruit-bodies of Pleurotus pulmonarius produced in the various substrates. The values ranged from 0.029% of vitamin B_1 in DE and EG (80%) to 0.89% in PM (40%), vitamin B_2 (0.16% in DE (80%) to 0.56% in TA (60%), vitamin B_3 (0.29% in TA (60%) to 0.95% in PM (40%), vitamin c (5.34% in PM (60%) to 7.82% in MI (80%).The fruit bodies of Pleurotus pulmonarius also contained different levels of phytochemicals which ranged; alkaloids (0.039% in TA (60%) to 0.094% in DE (60%), phenols (0.004% in TA (60%) to 0.06% in PM and EG (80%), Tannins (0.13% in MI(60%) to 0.85% in DE (60%), saponins (0.16% in PM (40%) to 0.43% in EG (40%), flavonoids 0.015 in MI (80%) to 0.53% in TA (40%) and sterols (0.015 in MI (80%) to 0.031 in TA (80%).Tropical mushrooms especially P. pulmonarius are rich in vitamins and phytochemical compositions. Attempts should be made to explore this species of Pleurotus commercially in Nigeria.

Key words: *Pleurotus pulmonarius*, Chemical composition, Vitamins, Supplements

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INTRODUCTION

Mushroom has been defined as the fruit of certain fungi analogous to apple on a tree [9]. Many fungi that form mushrooms exist in mycorrhizal relationship with trees, and this is one of the reasons why forests are often generous to mushroom hunters. Mushrooms have been universally recognized now as food and are grown on commercial scale in many parts of the world including Nigeria with high demand. The increased demand for mushrooms could be contingent upon the phenomenal rise in the unit costs of the conventional sources of animal proteins such as beef, pork, chicken and fish[3],[17], Mushrooms are cultivated for their taste, nutritional attributes and potential application in industries and they are useful for the preparation of medical concoctions and drugs [12], The importance of mushrooms in bioremediation and biodegradation has been reported by various scientists [1], [10], *Pleurotus species* have been used by the people all over the world for their nutritional, medicinal and other beneficial values [20], Mushrooms are good not only for immune-enhancement, but also to complement western chemotherapy and radiation therapy. Edible mushrooms are widely used as human food [8]. Mushrooms are very good dietary sources of food (referred to as neutraceuticals) contributing to the general well-being of humans. They are rich in unsaturated fatty acids, vitamins, phytochemical constituents and minerals, but low in calories [8]. They are known as the "meat" of the vegetable world [22]. Mushrooms are a low-calorie food usually eaten cooked or raw and as garnish to a meal. Dietary mushrooms are a good source of B vitamins, such as riboflavin, niacin and pantothenic acid. The vitamins content of many mushrooms have been investigated and results of such investigations show that they are rich in vitamins C, B_1, B_2, B_3 and vitamins D, [5], [17], Since vitamins are essential in the diet of man and conventional sources of vitamins are scarce [3], It is pertinent therefore that attempts made to increase the list of the sources of cheap vitamins, is not misdirected. Many mushrooms possess both excellent nutritional values

and pharmaceutical properties. There is increased interest in the consumption of mushrooms as food stems for their nutritional and therapeutic values and most mushrooms are very important nutritionally [16], The present investigations was focused on the phytochemicals and vitamin compositions of *Pleurotus pulmonarius* cultivated on bark of some indigenous fruit trees supplemented with agro-wastes. The results obtained from the study were used to discuss the essentials, therapeutic values and importance of edible mushrooms especially *Pleurotus pulmonarius.*

MATERIALS AND METHODS

Fresh and fleshy mushrooms (*Pleurotus pulmonarius)* grown on the different barks of fruit tree substrates supplemented with agro-waste (Fluted pumpkin wastes, at the ratio of 40% 60% and 80%) were harvested from the mushroom house of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike.

Preparation of samples for analysis

The fruit-bodies were prepared for analysis by drying themin the Selecta model oven at 104°C for four hours, following the method of [13], The dried specimens were broken into smaller pieces before grinding into fine powder using the Thomas Willey milling machine [17]. The dried and powdered samples were dispensed into air-tight bottles and they were all taken to the laboratory for the analysis.

DETERMINATION OF PHYTOCHEMICAL (BIOACTIVE COMPOUNDS) OF FRUIT BODIES OF P.PULMONARIUS STUDIED

Alkaloid determination

The determination of alkaloids in the samples *Pleurotus pulmonarius* fruit-bodies was carried out using the alkaline precipitation gravimetric method described by[11].

5g of the powdered sample was soaked in 20ml of 10% ethanoic acetic acid. The mixture stood for four hours at room temperature. Therefore, the mixture was filtered using Whitman filter paper. The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentration ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60^{0C} for 30 minutes, cooled in a desiccation and reweighed. The process repeated three more times and the average was taken .The weight of alkaloid was determined by the differences and expressed of weight of sample analyzed as shown below.

$$\% \text{ alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where; W_1= weight of filtrate, W_2=weight of filter paper + alkaloid precipitate

Flavonoid determination

The flavonoid content of the sample *P. pulmonarius* was determined by the gravimetric method as was described by [11], Five(5) g of the powdered sample was placed into a conical flask and 50ml of water and 2ml HCl solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture allowed to cool before it was filtrated through what-man filter paper.10 ml of ethyl acetate which contained flavonoid was recovered, while the aqueous layer was discarded. A pre weighed what man filter paper was used to filter the second (ethyl- acetate layer), the residue was then placed in an oven to dry at 60 °C. It was cooled in a desiccators' and weighed. The quantity of flavonoid was determined using the formula.

$$\% \text{ flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where; W_1= weight of empty filter paper, W_2= weight of paper + flavonoid extract.

3.7.3 Determination of phenols.

The concentration of phenols in the fungi *P. pulmonarius* was determined using the folin-cio Caltcean colorimetric method described by [11], A sample of 0.2g of the powdered samples were each added into different test tubes and 10mls of methanol were added to each of them and they were shaken thoroughly, the mixtures were left to stand for 15 minutes before being filtered using What man filter paper. I ml of the extract was placed in a text-tube and I ml of Folin–ciocaltean reagent in 5ml of distilled water were added and color were allowed to develop for about 1 to 2

hours at room temperature. The absorbance of the development colour was measured at 760nm wave each. The process was repeated three more times and an averaged taken. The phenol content was calculated thus.

% phenol= 100/W x AU /AS x C/100 x VF/ VA x D

Where; W=weight of sample analyzed, AU= absorbance of test sample, AS= absorbance of standard solution, C= concentration of standard in mg/ml, VF=total filtrate volume, VA= Volume of filtrate analyzed, D= dilution factor where applicable

Determination of saponins
The saponin content of the sample was determined by double extraction gravimetric method [11].

5grams of the powered samples were mixed with 50ml of 20% aqueous ethanol solution in a flask. The mixtures were heated with periodic agitation in water bath for 90 minutes at 550^{0C}. They were then filtered through what man filter paper. The residue were extracted with 50ml of 20% ethanol and both extract were poured together and the combined extracted were reduced to about 40ml at 90^{0C} and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer become clear in color. The saponins were extracted, with 60ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. They were dried at 60 °C in the oven and reweighed after cooling in desiccators. The process was repeated three more times to get an average. Saponin contents were determined by difference and calculated as a percentage of the original sample thus.

$$\% \text{ Saponin} = \frac{W_2 - W_1}{\text{Wt of sample}} \times \frac{100}{1}$$

Where, W_1= weight of evaporating dish, W_2= weight of dish + sample

Determination of steroid
The steroid content of the samples *P. pulmonarius* were determined using the method described by [11], Five (5) grams of the powdered sample were hydrolyzed by boiling in 50ml of hydrochloric acid solution for about 30 minutes. It was filtered using what man filter paper.

The filtrates were transferred into separating funnels. Equal volume of ethyl acetate were added to them, mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) was used while the aqueous was discarded. The extracts were dried at 100 °C for 5 minutes in a steam bath. They were then heated with concentrated amyl alcohol to extract the steroids. The mixture becomes turbid and a reweighed what man filter paper was used to filter the mixtures properly. The dry extract were then cooled in desiccators and reweighed. The process was repeated two more time and an average was obtained. The concentration of steroid was determined and expressed as a percentage thus

$$\% \text{ steroid} = \frac{W_2 - W_1}{\text{Wt of sample}} \times \frac{100}{1}$$

DETERMINATION OF SOME VITAMINS OF MUSHROOM (*P. PULMONARIUS)*
The spectrophotometric method by [4],was employed in the determination of vitamin contents.

Determination of riboflavin (Vit. B_2)
Fumes of each sample was extracted with 100mililitres of 50% ethanol solution shaken for 1 hour and was filtered. 10mililitres of 30% hydrogen peroxide (H_2O_2). The mixture was allowed to stand on a steam bath for 30minutes, after such 2 milliliters of Na_2SO_4 solution was added. It was dilated to 50mililitres with distilled water and its spectrophotometer at 510nm wavelength. Meanwhile, a standard riboflavin solution was prepared and diluted, 10 milliliters portion of it was allowed to stand on a steam bath for 30minutes, and after such 2 milliliters of Na_2SO_4 solution was added. It was dilated to 50mililitres with distilled water and its spectrophotometer at 510nm wavelength. Meanwhile, a standard riboflavin solution was prepared and diluted, 10 milliliters portion of it was analyzed as discussed above. The reading were taken with the reagent blank at zero, the formula below was used.

Riboflavin mg/100 = $\frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vf}{Va} \times D$

Where, W=weight of sample analyzed, Au= absorbance of the test sample, As= absorbance of the standard solution, Vf= total volume of filtrate, Va= volume of filtrate analyzed, C= concentration of the standard, D= dilution factor where applicable

***Vitamin B_1 (Thiamine*)**

The method of [19], was used for vitamin B1 determination. Two (2g) of sample was weighed out and 50ml of alcoholic NaOH was added and allowed to stand for few minutes before being filtered out. Ten (10ml) of filtrate was measured out and 10ml of potassium dichromate added. The absorbance was read at 430nm.

Calculation of vitamin B1 was done as follows

$$\text{Weight of sample} = \frac{100}{as} \times au \times C \times \frac{VF}{Va} \times D$$

Where: au = absorbance of sample as = absorbance of stand
C = conc. of standard (mg/l) VF = total volume of extract
Va = volume of extract analyzed D = dilution factor where applicable

Determination of niacin (Vit.B_3)

5grams of the sample was treated with 50 milliliters of 1N sulphuric acid and shaken for 39 minutes, 3 drops of ammonia solution was added to the sample and filtrate into a 50mililitres volumetric flask and 5mililitres of potassium cyanide was added. This was acidified with 5 milliliters of 0.02N sulphuric acid and absorbance was measured in the spectrometer at 470nm wavelength.

A standard niacin solution was prepared and diluted. 10mililitres of the solution was analyzed as discussed above. The readings were made with reagent blank at zero. The formula below was used.

Niacin mg/100g = $\frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vf}{Va} \times D$

Where, W= weight of sample analyzed, Au= absorbance of the test sample, As= absorbance of the standard solution, Vf= total volume of filtrate, Va= volume of filtrate analyzed, C= concentration of the standard solution, D= dilution factor where applicable

Vitamin C Determination

The method of [19],was used for vitamin c determination. Five (5g) of the sample was used for the determination. The Vitamin C content was calculated based on the relationship that 1ml 0.01m CuSO4 = 0.88mg Vitamin C.

Therefore vitamin C Mg/100g

$$= \frac{100}{W} \times 0.88 \times (T.B) \times \frac{vt}{VA}$$

Where: W = Weight of sample T = Titre value of sample
B= Titre value of blank Vt = Total extract volume
Va = volume of extract titrate'

RESULTS AND DISCUSSION

The result of the vitamin compositions of the mushrooms is summarized in Table 1.

The result showed that the vitamin compositions of the mushrooms produced or cultivated on barks of some indigenous fruit trees supplemented with agro-wastes ranged as follows; vitamin B_1(0.029% in *D. edulis* and E. *guineensis* (80%) to 0.89% in *P. macrophylla* (40%), vitamin B_2 (0.16% in *D. edulis* (80%) to 0.56% in *T. africana* (60%), vitamin B_3 (0.29% in *T. africana* (60%) to 0.95% in *P. macrophylla* (40%), vitamin C (5.34% in *P. macrophylla* (60%) to 7.82% in *M. indica* (80%).

Table 1. Vitamin compositions of *P. pulmonarius* produced on barks of fruit trees supplemented with agro-wastes

Parameters (mg/100g)	Inclusion levels of substrates + supplements (%)	TA	MI	DE	PM	EG
(Vit.B_1)	40	0.68±0.001 ^{b}	0.52±0.001 ^{c}	0.43±0.002 ^{d}	0.89±0.000^{a}	0.35±0.001 ^{e}
	60	0.38±0.001 ^{b}	0.48±0.001 ^{a}	0.35±0.000 ^{b}	0.44±0.01 ^{ab}	0.00
	80	0.45±0. 002^{c}	0.85±0.001 ^{a}	0.29±0.000 ^{d}	0.76±0.005 ^{b}	0.29±0.005 ^{d}
(Vit. B_2)	40	0.26±0.003 ^{c}	0.29±0.000^{b}	0.31±0.003 ^{b}	0.37±0.001 ^{a}	0.27±0.001 ^{c}
	60	0.56±0.000 ^{a}	0.25±0.000 ^{b}	0.14±0.005 ^{c}	0.26±0.001^{b}	0.00
	80	0.27±0.002 ^{b}	0.47±0.01 ^{a}	0.16±0.00 ^{c}	0.24±0.000 ^{b}	0.18±0.000 ^{c}
Niacin (B_3)	40	0.48±0.000 ^{d}	0.64±0.001 ^{c}	0.63±0.00 ^{c}	0.95±0.001 ^{a}	0.77±0.001 ^{b}
	60	0.29±0.000 ^{d}	0.55±0.001 ^{b}	0.48±0.000 ^{c}	0.68±0.001 ^{a}	0.00
	80	0.47±0.002 ^{d}	0.78±0.000 ^{b}	0.47±0.002 ^{d}	0.89±0.001^{a}	0.69±0.000 ^{c}
(Vit C.)	40	6.74±0.000 ^{c}	7.46±0.40 ^{b}	6.17±0.01 ^{d}	5.46±0.000 ^{e}	7.53±0.08 ^{a}
	60	6.09±0.005 ^{b}	6.71±0.01 ^{a}	5.76±0.04 ^{c}	5.34±0.06 ^{d}	0.00
	80	6.71±0.01 ^{b}	7.82±0.02 ^{a}	5.87±0.03 ^{d}	6.27±0.03 ^{b}	6.19±0.005 ^{c}

$^{a-b}$ *Means in the same row with different super scripts are significantly different (p <0.05) while means in the same row with similar superscripts are not significantly different (p>0.05)*

TA = Treculia Africana, MI= Mangifera indica, DE= Dacryodes edulis, PM= Pentaclathra macrophylla, EG= Elaeise guineensis

Table 2. Phytochemical compositions of *P. pulmonarius* produced on barks of fruit trees supplemented with agro-wastes

Parameters (%)	Inclusion levels of substrates + supplements (%)	TA	MI	DE	PM	EG
ALKALOIDS	40	0.054±0.00^{d}	0.077±0.001 ^{c}	0.130±0.0^{a}	0.086±0.05^{b}	0.07±0.00 ^{c}
	60	0.039±0.00^{d}	0.053±0.001 ^{c}	0.094±0.0^{a}	0.075±0.00^{b}	0.00
	80	0.061±0.01^{c}	0.06±0. 00 ^{c}	0.11±0.01^{a}	0.069±0.00^{c}	0.075±0.00^{b}
PHENOLS	40	0.008±0.00 ^{c}	0.019±0.001^{b}	0.015±0.00^{b}	0.044±0.00^{a}	0.009±0.00^{c}
	60	0.004±0.00 ^{c}	0.012±0.00 ^{b}	0.016±0.0^{b}	0.029±0.00 ^{a}	0.00
	80	0.054±0.00^{b}	0.025±0.02^{c}	0.018±0.0^{d}	0.063±0.00^{a}	0.06±0.00 ^{a}
TANNINS	40	0.49±0.00 ^{a}	0.23±0.00 ^{d}	0.35±0.01^{b}	0.14±0.00 ^{e}	0.28±0.00 ^{c}
	60	0.51±0.01 ^{b}	0.13±0.00 ^{c}	0.85±0.01^{a}	0.21±0.00^{d}	0.00
	80	0.58±0.01 ^{a}	0.18±0.00^{e}	0.41±0.01 ^{b}	0.19±0.00 ^{d}	0.31±0.01 ^{c}
SAPONINS	40	0.28±0.01^{b}	0.31±0.15^{b}	0.24±0.01^{c}	0.16±0.00^{d}	0.43±0.00 ^{a}
	60	0.19±0.00 ^{c}	0.25±0.00 ^{b}	0.19±0.00 ^{c}	0.32±0.00 ^{a}	0.00
	80	0.26±0.01^{b}	0.23±0.015^{c}	0.22±0.00 ^{d}	0.25±0.00 ^{b}	0.39±0.01^{a}
FLAVONOID	40	0.53±0.00 ^{a}	0.046±0.001^{e}	0.18±0.01 ^{c}	0.11±0.01^{d}	0.31±0.00^{b}
	60	0.28±0.01 ^{b}	0.016±0.00 ^{d}	0.26±0.00 ^{c}	0.30±0.00 ^{a}	0.00
	80	0.37±0.01^{a}	0.015±0.00 ^{e}	0.14±0.00^{d}	0.29±0.00 ^{c}	0.32±0.00 ^{b}
STEROLS	40	0.032±0.02^{d}	0.046±0.01^{b}	0.039±0.0^{c}	0.035±0.01^{c}	0.195±0.003^{a}
	60	0.029±0.01^{b}	0.016±0.00 ^{d}	0.02±0.01^{c}	0.033±0.02^{a}	0.00
	80	0.031±0.00 ^{a}	0.015±0.001^{d}	0.027±0.0^{b}	0.022±0.04^{c}	0.028±0.02^{b}

$^{a-b}$ *Means in the same row with different super scripts are significantly different (p <0.05) while means in the same row with similar superscripts are not significantly different (p> 0.05*

**See meaning of abbreviations on Table 1*

The results have showed that the fruit-bodies of *P. pulmonarius* contain vitamins B_1 (thiamin), vitamins B_2(riboflavin), and vitamins B_3 (niacin) and vitamins C (ascorbic acid) in varying quantities. The vitamin contents of the mushroom are supported by the reports of [17], *Pleurotus* mushrooms are rich in vitamins C, B_1, B_2, B_3 and vitamins D. The vitamins however showed significant differences as affected by the different levels of supplements. Supplemented *P. macrophylla* (40%) significantly ($p<0.05$) boosted the vitamins (B_1 and B_3) content of the mushroom fruit-bodies, whereas ascorbic acid showed best amount with *M. indica* (80%) supplemented substrates. The ranges of values of niacin recorded in this study were lower than the amount reported by [14]. The supplemented *T. Africana* (60%)significantly ($p<0.05$) produced highest amount of riboflavin. These vitamins are very vital for body nourishment and are essential in the diet of man, hence can be produced from cheap and available substrates supplemented with agro-wastes. However, it has been stated that conventional sources of vitamins are scarce [3]. This result has revealed that, the different agro-wastes used as supplements significantly influenced the vitamin contents of mushroom (*P. pulmonarius).*

The result of the phytochemical compositions of the mushrooms is summarized in Table 2.

The result showed that the phytochemical compositions of the mushrooms produced or cultivated on barks of some indigenous fruit trees supplemented with agro-wastes ranged as follows; alkaloids (0.039% in *T. africana* (60%) to 0.094% in *D. edulis* (60%), phenols (0.004% in *T. africana* (60%) to 0.06% in *P. macrophylla and E. guineensis* (80%), Tannins (0.13% in *M. indica* (60%) to 0.85% in *D. edulis* (60%), saponins (0.16% in *P. macrophylla* (40%) to 0.43% in *E. guineensis* (40%), flavonoids 0.015 in *M. indica* (80%) to 0.53% in *T. africana* (40%) and sterols (0.015 in *M. indica* (80%) to 0.031 in *T. africana* (80%).

The results are means of triplicate determinations on dry weight basis ± standard error. The results have revealed that fruit-bodies of *P. pulmonarius* contain alkaloids, phenols, tannins, saponins, flavonoids and steroids in varying quantities. These metabolites in the mushroom studied have proved it to be of high medicinal importance. This is supported by the report that the presence of alkaloids, phenols and phenolic compounds in certain plants has been associated with their anti-microbial properties [15],Mushrooms have been discovered to have therapeutic values. [7],reported that mushrooms have anti-tumoral, anti-cancer, anti-cholesterol and anti-hemorrhage effects. Most bioactive compounds which play essential roles in human and animal physiology have been found in many mushrooms. *P. macrophylla* and *E. guineensis* (80%) yielded significantly ($p<0.05$) highest amount of phenols among the substrates. The values of phenols in this study are similar to that report for *Schizophyllum commune* by [16].The presence of these bioactive compounds in the mushroom produce in different levels of substrates is of great benefit to human health. However, the success in growing these mushrooms for high phenolic compounds with *P. macrophylla* and *E. guineensis* (80%) substrates suggests that it has medicinal potentials. According to [21], the plants widely used in traditional medicine, contain bioactive compounds which are precursors for useful drug synthesis. They are useful for the preparation of medical concoctions and drugs [12], *D. edulis* (60%) was significantly ($p<0.05$) highest in tannin content when compared with other substrates. The amount of tannins obtained in this study is not far from that reported in *S. commune* an edible mushroom [16].Tannins inhibit pathogenic fungi and also reduce the rate of grazing on plants by animals that feed of plants. They also affect many human physiological activities such as stimulation of phagocyte cells, host-mediated anti-tumor activities and a wide range of anti-infective actions [6].This study has revealed that *T. Africana* and *M. indica* when supplements are used on them can produce good mushrooms with significant ($p<0.05$) high amount of alkaloids, flavonoids, sterols and low tannins indicating excellent quality of mushrooms. Flavonoids have been reported to be useful in the treatment of some physiological disorders and diseases. This study has revealed that supplemented *T. africana* has been found to be good substrate for production of *Pleurotus pulmonarius* with high flavonoids content. And this work is similar to the work of [18], who detected large quantities of flavonoids in *Pleurotus tuber-regium*. The amount of saponins recorded in this work is similar to the amount reported for *S. commune* by [16]. Many drugs are synthetic sterols and also serve as an anabolic hormone used to promote muscle growth.

CONCLUSION

The result has revealed that, substrates supplementations could be very vital in the production of mushrooms with high amount of the bioactive substances. The mushroom contains appreciable levels of vitamins and phytochemicals in all the substrates and their supplementations. However there is no clear cut influence of the supplement with regards to the preferred supplement and level that would give the best of bioactive substances. With the report the report that the presence of alkaloids, phenols and phenolic compounds in certain plants has been associated with their anti-microbial properties [15]. The *P. pulmonarius* produced from the different substrates supplementations is still medicinal.

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