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Evaluation of Lignocellulosic Enzymes Profile of *Pleurotus sajor-caju* Grown on Selected Agro-Industrial Wastes

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Abstract *Pleurotus sajor-caju* is an important edible mushroom gaining popularity in recent years because of its high nutritional value and ability to grow on diverse agricultural wastes. Certain enzymes have been associated with their pattern of medium degradation, growth or development. Cultivation of *P. sajor-caju* was carried out using three agricultural wastes (rice straw, sawdust, rice bran) and an industrial waste (Brewers spent grain). The enzyme production from *P. sajor-caju* cultivated on these agro-industrial wastes was monitored for a period of sixty (60) days. *P. sajor-caju* cultivated on the different substrates were analyzed for proximate composition and toxicological effects. Cultivation of *P. sajor-caju* on rice straw had the least cultivation period (28 to 30 days), while *P. sajor-caju* cultivated on rice bran lasted for 45 to 48 days making these two substrates the most suitable for the growth of *P. sajor-caju.* Laccase had the highest enzyme activity on rice bran and sawdust (0.422µmol/min/ml and 1.44µmol/min/ml), manganese peroxidase production was the highest on rice straw (1.1063µmol/min/ml) and cellulase was the most active enzyme on brewers spent grain (0.8843µmol/min/ml). The proximate composition of *P. sajor-caju* cultivated on the Agro-industrial waste used showed that mushrooms cultivated on sawdust had the highest tannin (6.72 mg/100g), phytate (162.48mg/100g) and alkaloid content (3.7%), while mushrooms cultivated on brewers spent grains had a lower tannin, phytate and alkaloid and those cultivated on rice straw had the lowest antinutritional contents. Toxicity study on the effect of *Pleurotus sajor-caju* on the liver function indicator showed that the experimental feeding of albino rats with *Pleurotus sajor-caju* had no adverse effects on the enzyme indicators [Alanine Amino Tranferase (ALT), Aspartate Amino Transferase (AST), Gamma Glutamyl Transferase (GGT) and Alkaline Phophatase (ALP)] for liver function.

Keywords: *Pleurotus sajor-caju, Lignocellulosic enzymes, Antinutrient composition*

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1. Introduction

Mushroom is a fleshy, spore-bearing fruiting body of a fungus produced above the ground on soil or on its food source. Mushrooms are not plants and therefore do not undergo photosynthesis, their mode of nutrition is by producing different range of enzymes that can breakdown complex substances after which they are able to absorb the soluble substances formed [1].

The genus Pleurotus (Pleurotaceae, higher Basidiomycetes) comprises a group of edible ligninolytic mushrooms with medicinal properties and important biotechnological and environmental applications [2]. *Pleurotus* spp are promising as medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulation activities [2].

P. sajor-caju also known as Dhingri have ability to grow on diverse agricultural wastes. Poppe [3] reported that there are about 200 kinds of wastes suitable for production of edible mushrooms. Various agricultural wastes rich in cellulose are being used as substrates for cultivation of Dhingri mushrooms [4]. Most of all, *Pleurotus* spp. can utilize various kinds of substrate materials compared to any other mushrooms. *Pleurotus* species require a temperature of 20-30 °C both for its vegetative growth and reproductive phase in natural habitat [1]. Mushrooms reduce agricultural solid wastes, are highly efficient in bioremediation and have many medicinal applications [5,6,7,8,9].

Bioconversion of lignocellulosic wastes through cultivation of *Pleurotus* species converts renewable resources for the production of edible, protein-rich food that will sustain food security for people in developing countries [10]. Cellulases are enzymes that break the glucosidic bonds of cellulose microfibrils, releasing oligosaccharides, cellobiose and glucose [11]. Cellulase is produced by a vast and diverse fungus population, such as the genera *Trichoderma*, *Chaetomium*, *Penicillium*, *Aspergillus*, *Fusarium* and

Phoma; aerobic bacteria, such as *Acidothermus*, *Bacillus*, *Celvibrio*, *Pseudomonas*, *Staphylococcus*, *Streptomyces* and *Xanthomonas*; and anaerobic bacteria, such as *Acetovibrio*, *Bacteroides*, *Butyrivibrio*, *Caldocellum*, *Clostridium*, *Erwinia*, *Eubacterium*, *Pseudonocardia*, *Ruminococcus* and *Thermoanaerobacter* [12]. Microorganisms are the rich sources of xylanases, produced by wide diverse genera and species of bacteria, actinomycetes and fungi [13]. The present study aim to investigate the activities of cellulase, laccase, manganase peroxidase and xylanase produced during the cultivation of *Pleurotus sajor- caju* on rice straw, sawdust, brewers spent grain and rice bran. The study also reports the antinutrient properties and toxigenicity of *Pleurotus sajor-caju* cultivated on the different agricultural wastes.

2. Materials and Methods

2.1. Source of Samples

Spawn of *Pleurotus sajor-caju* was obtained from Forest Research Institute of Nigeria (FRIN), Ibadan. Brewers spent grains was collected from International breweries, Ilesha, while the agricultural wastes (Rice straw, rice bran, saw dust etc.) was obtained in Ijan Ekiti, Ekiti State.

2.2. Substrate Preparation

Five hundred (500) grams of each substrate (rice straw, rice bran, brewers spent grain and saw dust) was weighed, soaked in 1 liter of distilled water (pH 7), sealed aseptically in polythene bags (stuffing). However, rice straw was chopped into smaller sizes before it was weighed. The polythene bags were then sterilized in an autoclave for an hour at 121°C. After sterilization, bags were allowed to cool before inoculating with spawn.

2.3. Mushroom Production

Spawn of 10 % or more of the dry weight of the substrates was aseptically added to the sterilized substrates and hermetically sealed. Bags were punctured at the base for minimum aeration. Inoculation of the bags, i.e. spawning was carried out through multilayered spawning. The inoculated bags were kept in a dark cupboard and covered with black polythene till the cottony growth proliferates. When the substrate was completely covered with the white cottony mycelia growth, the polythene bags were removed, perforated and moved to rooms where air and light could penetrate for the initiation and subsequent development of fruiting bodies. Fully grown mushrooms were harvested by gently breaking mushrooms from substrate and the bags were wetted lightly with water. This lasted for about 5 weeks.

Observations on period of spawn run, appearance of pinhead, maturation of fruiting bodies were recorded up to fourth flush to calculate the corresponding biological efficiency. Biological efficiency was determined by the ratio of total fresh weight (g) of mushrooms obtained from four flushes to dry weight (g) of substrate and expressed as percentage. Samples of substrate used for cultivation of *P. sajor-caju* were picked from day 0 of cultivation till day 60 at an interval of 5 days.

2.4. Enzyme Extraction and Determination of Enzymatic Activity

Substrates were homogenized in 25 ml phosphate buffer (pH 7.0, 1 g of sample + 5 ml buffer). Homogenate was centrifuged at 6000 rpm for 15 minutes at 4°C, supernatant was filtered with Whatman filter paper and filtrate was used as crude enzyme solution.

2.4.1. Estimation of Cellulase Activity

Cellulase activity was assayed using Dinitrosalicyclic acid (DNSA) reagent [14] by estimation of reducing sugars released from Carboxyl Methyl cellulose (CMC) solubilized in 0.05 M phosphate buffer at pH 8 [15]. The crude enzyme was added to 0.5 ml of 1 % CMC in 0.05M phosphate buffer and incubated at 50°C for 30 min. After incubation, reaction was stopped by addition of 1.5 ml of DNSA reagent and boiled at 100°C in water bath for 5 min. Sugars liberated were determined by measuring absorbance at 540 nm. Cellulase production was estimated by using glucose calibrated curve [16]. One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1 µmol of glucose per minute under standard assay conditions.

2.4.2. Estimation of Xylanase Activity

Xylanase activity was assayed at 70°C using 1% (w/v) beechwood xylan at pH 9.0. The reducing sugars released were determined using xylose as standard [15,17]. The reaction mixture consisted of 400 µl of the xylan and 100 µl of culture supernatant for 10 minutes at 70°C. DNS reagent was added (500 µl), after which the mixture was boiled for 5 minutes and cooled in ice. The resultant color intensity was estimated at 549 nm using a visible spectrophotometer. One unit (U) of xylanase activity was defined as the amount of enzyme required to liberate 1 µmol of xylose per minute under assay conditions. The substrate incubated with 50 mM Tris buffer (pH 9.0) under conditions was used as control.

2.4.3. Estimation of Laccase Activity

Activity of laccase was determined according to a modified method of Bourbonnais and Paice [18]. This was done by monitoring spectrophotometrically the change in absorbance at 420 nm (A_{420}) related to the rate of oxidation of 1 mM 2,2'-azino-bis-[3-ethylbenzthiazoline-6-sulfonate] (ABTS) in 1 mM Tris-HCl buffer (pH 7.0). Assays were performed in 1 mL cuvettes at room temperature with 750 µL ABTS and 250 µL of enzyme extract. One unit of laccase activity was defined as the amount of enzyme that leads to the oxidation of 1 µmol of ABTS per minute with a molar extinction for the ABTS radical cation (the reaction product) of $\varepsilon_{420\ nm}$= 36000 M^{-1} cm^{-1}. Laccase activity was expressed as unit per millilitre (U/mL). The enzyme activity was calculated using the expression:

$$Activity = \frac{\left(\begin{array}{l} Absorbance / minute \\ x\ Total\ volume\ of\ mixture \end{array}\right)}{\left(\begin{array}{l} Total\ time\ x\ Extinction\ coefficient \\ x\ Volume\ of\ enzyme \end{array}\right)}.$$

2.4.4. Estimation of Manganese Peroxidase Activity

Manganese peroxidase (MnP) activity was determined by measuring the oxidation of manganic malonic complex. Assay mixtures (1.3 ml) contained sodium malonate buffer (pH 4.5, 50 mM), $MnSO_4$ (50 mM), H_2O_2 (0.1 mM) and 0.05 ml of MnP sample. One unit of MnP activity was defined as 1mol product formed per minute. All enzyme assays were carried out in a UV-Vis spectrophotometer.

2.5. Protein Content Determination

Protein content was determined by the method of Bradford [19] using Bio-rad protein assay (dye reagent). In this assay, 200 µl of the diluted dye was pipetted into 10 µl of sample solution. The mixture was then incubated at room temperature for 15 minutes to allow proper colour development. The absorbance was measured at 595 nm against a blank (control) containing essentially the same mixture but with the sample solution substituted for an equal volume of distilled water. To derive a protein standard curve, a stock solution of 0.2 mg/ml bovine serum albumin (BSA) was prepared and diluted serially to concentrations varying from 0.02 to 0.01 mg/ml. Two hundred microlitres (200 µl) of Bradford reagent was added to each solution and the mixtures were incubated for 15 minutes. Afterwards, the absorbance was measured at 595 nm to obtain the standard curve [19].

2.6. Determination of Antinutrient Properties

The antinutrient properties observed were phytate, oxalate, tannin and alkaloid content of the mushrooms samples.

2.6.1. Determination of Phytate

Four grams (4 g) of the powdered sample was soaked in 100 ml of 2 % HCl for 3 hrs and then filtered through a No 1 Whatman filter paper. Twenty five millilitre (25 ml) was taken out of the filtrate and placed inside a conical flask after which 5 ml of 0.3 % ammonium thiocyanate solution was added as indicator and 53.5 ml of distilled water to give it the proper acidity and titrated against iron (III) chloride solution that contains about 0.0019 g of iron per milliliter until a brownish yellow coloration which persist for 5 minutes indicating a positive result is observed, a negative result is indicated by absence of brown coloration as described by Day and Underwood [20], with slight modifications.

2.6.2. Determination of Oxalate

One gram (1g) of powdered sample was soaked in 75ml of 1.5 N. H_2SO_4 for 1 hr and then filtered through a No 1 Whatman filter paper. Twenty –five milliliters (25 ml) will be taken out of the filtrate, placed inside a conical flask and titrated hot about 80-90°C against 0.1 M $KMnO_4$ until a pink color that persist for 15 seconds which indicates a positive test for oxalate was observed, absence of pink coloration indicates a negative result [20] with slight modifications.

2.6.3. Determination of Alkaloids

Five grams (5 g) of the powdered sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and allowed to stand for 4 minutes, this was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide is added drop-wisely to the extract until precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue (alkaloid) was dried and weighed as described by Harbone [21], with slight modification.

3. Results

3.1. Production of Mushrooms on Different Wastes

Table 1 shows the result of the days for cultivation of mushrooms on the agro-industrial wastes used. Spawn run lasted for 24 to 26 days in rice straw, 26 to 27 days in saw dust, 29 to 32 days in brewers spent grains and 30 to 32 days in rice bran. However, pin head formation lasted between 25 to 45 days. Fruiting bodies of *P. sajor-caju* cultivated on the different wastes appeared within 28 to 48 days. Mushrooms cultivated on rice straw and saw dust showed fruiting bodies at 28 to 30 days and 30 to 32 days respectively which was earlier than those cultivated on brewers spent grains while mushrooms cultivated on rice bran had the longest number of days to produce its fruiting bodies.

From Table 2, it was observed that *P. sajor-caju* thrived on the agro-industrial wastes. The highest yield and biological efficiency were observed in saw dust (112±0.12 g, 109.7 %) all through the flushes followed by those cultivated on rice straw (96.0±0.15 g, 90 %) and brewers spent grains (92.6±0.15 g, 85.76 %). However, mushrooms cultivated on rice bran had the lowest yield and biological efficiency (56±0.02 g, 37.61 %) among the substrates used.

Table 1. Days for initiation of spawn, pinheads and fruiting bodies

Substrates	Spawn run (Days)	Pinhead formation (Days)	Fruiting body formation (Days)
Rice straw	24-26	25-28	28-30
Saw dust	26-27	28-31	30-32
Brewers spent grain	29-32	35-38	37-40
Rice bran	30-32	42-45	45-48

Table 2. Yield of fresh mushrooms

Substrates	1st flush (g)	2nd flush (g)	3rd flush (g)	4th flush (g)	Biological efficiency (%)
Saw dust	112.0±0.12c	92.0±0.11cd	70.4±0.12d	54.7±0.16b	109.7cd
Rice straw	96±0.15bc	76.5±0.23c	57±0.16c	40.5±0.12a	90.0c
Brewers spent grain	92.6±0.15b	68.2±0.11bc	52.5±0.15c	44±0.09a	85.76c
Rice bran	56.0±0.02a	38.65±0.09a	18.20±0.11a	-	37.61a

Means for each treatment with the same alphabet in each row are not significantly different at 5% level of significance ($p \leq 0.05$).

There was no significant difference among the yields of *P. sajor-caju* cultivated on the agro-industrial wastes in the first flush. For the second flush, there was no significant difference between yields from sawdust, rice straw and brewers spent grains although yield from sawdust, rice straw and rice bran were significantly different. In the third flush, yields of *P. sajor-caju* from rice bran were significantly different from yields of *P. sajor-caju* from other agro- industrial wastes. *P. sajor-caju* cultivated on rice bran did not thrive well in the fourth flush (Table 2).

3.2. Enzyme Production during Mushroom Cultivation

The production of enzymes by mushroom in rice straw, rice bran, sawdust and brewer spent grain is shown in Figure 1, Figure 2, Figure 3 and Figure 4 respectively. Generally, the activities of cellulase, xylanase, manganese peroxidase and laccase were duration of culture dependent. The activities of the enzymes increased over time. All the enzymes (cellulase, xylanase, manganese peroxidase and laccase) assayed for was tremendously increased in rice straw although with a slight decrease in the activity of cellulase, laccase and manganese peroxidase on day sixty (60) of the cultivation. Manganese peroxidase had the highest activity on rice straw (Figure 1).

On rice bran, the activities of enzyme followed similar pattern (Figure 2). There was a gradual increase in the activities of enzymes assayed for in the first fifteen days followed by decrease in enzyme activities then a steady increase in the activity of the enzymes. Laccase and manganese peroxidase had the highest activity among the enzymes assayed for on rice bran.

Laccase and manganese peroxidase had the highest activity on sawdust (Figure 3). There was an increase in the activity of laccase all through the period of cultivation of *Pleurotus sajor-caju.* On brewers spent grain substrate (Figure 4), cellulase had the highest activity among the enzyme assayed for with a slight decrease in activity at the latter days of cultivation. Xylanase, manganese peroxidase and laccase activity increased with increase in the days of cultivation. Laccase and manganese activities were moderately produced with time in substrates.

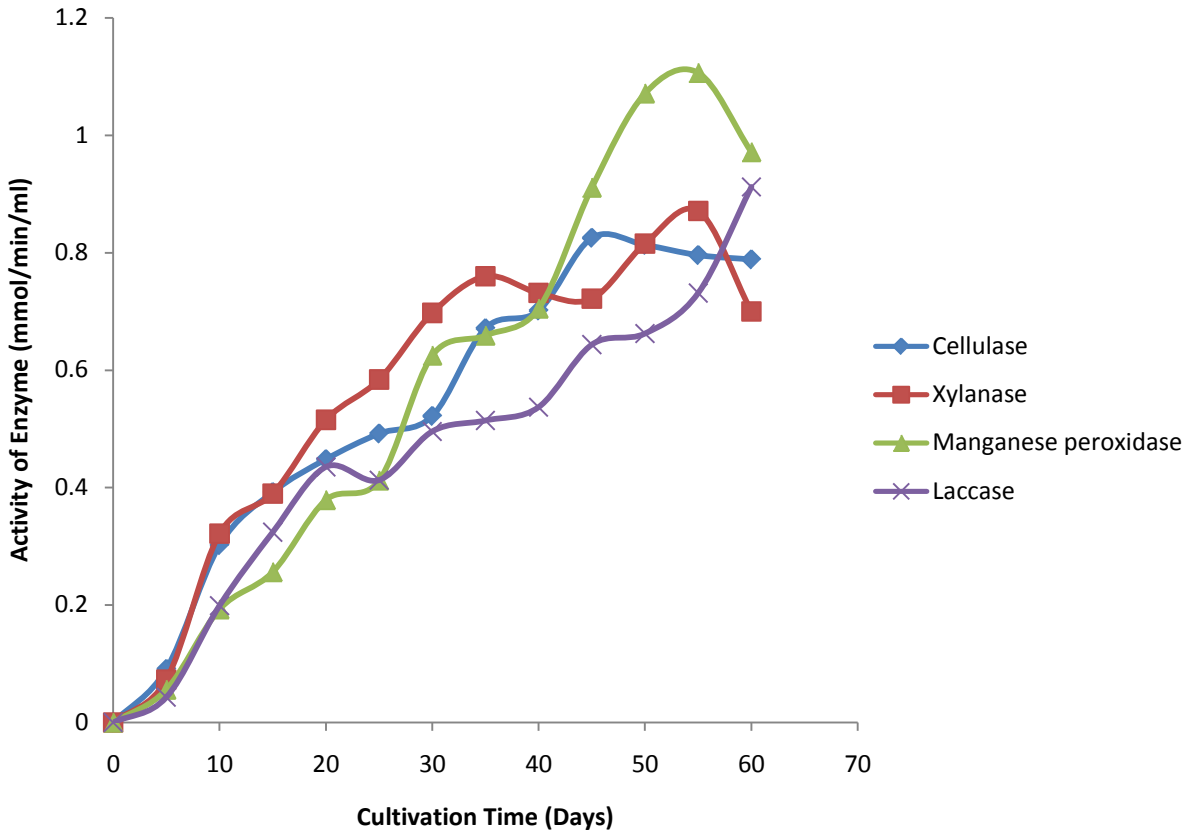


Figure 1. Time-course of activities of enzymes produced from *Pleurotus sajor-caju* grown on rice straw

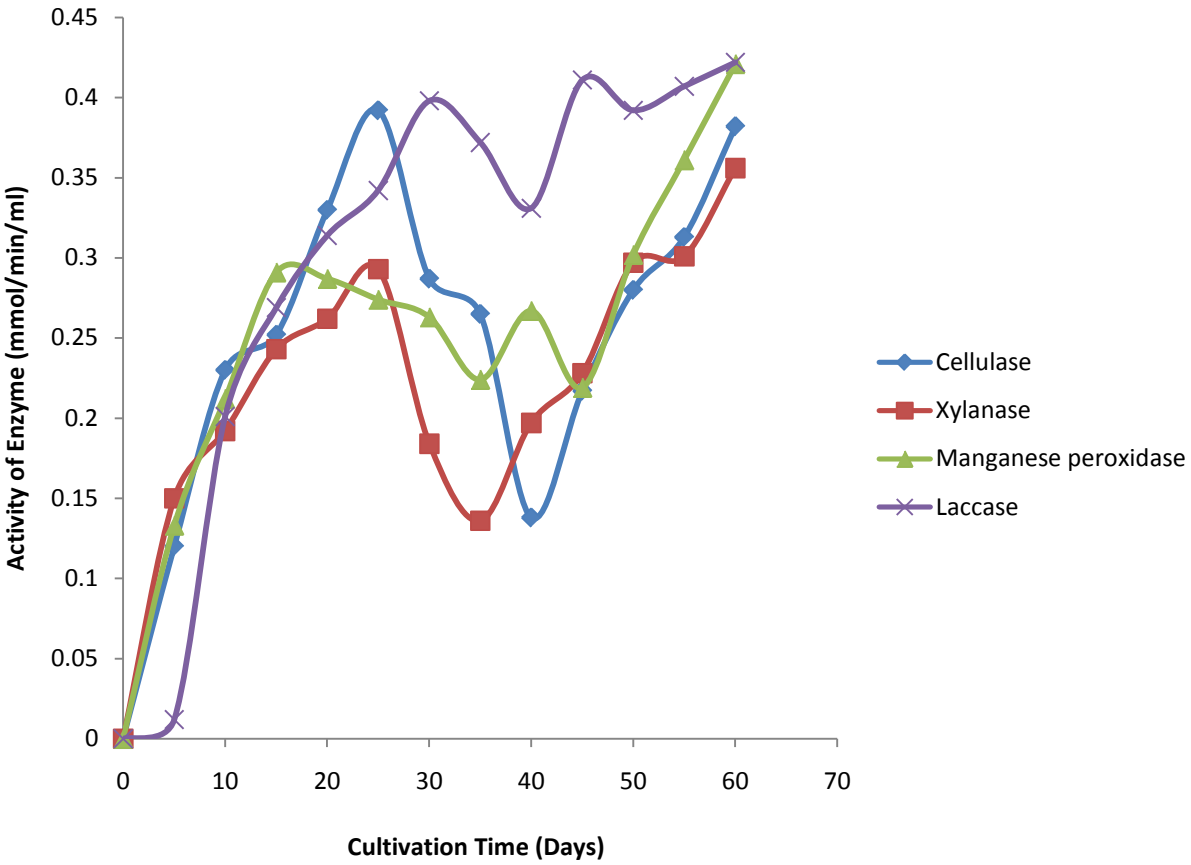


Figure 2. Time-course of activities of enzymes produced from *Pleurotus sajor-caju* grown on rice bran

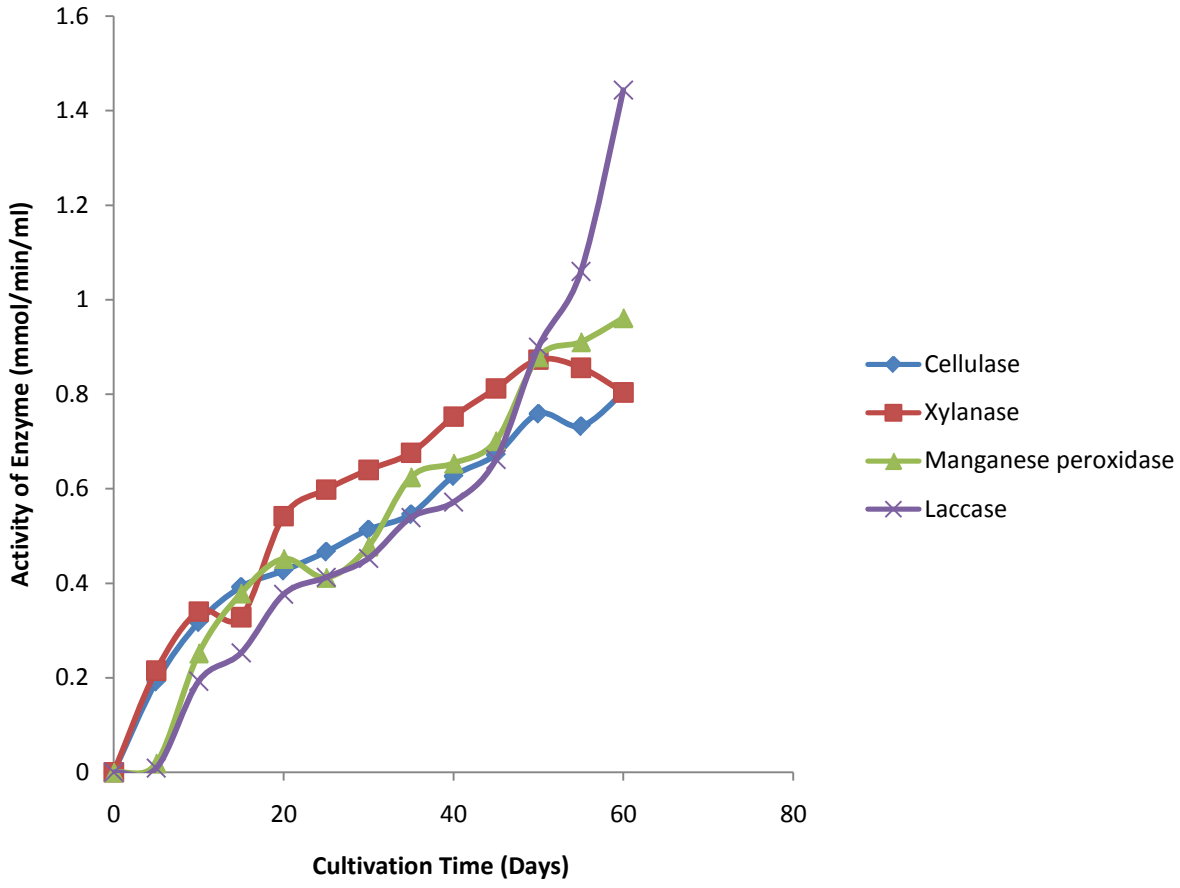


Figure 3. Time-course of activities of enzymes produced from *Pleurotus sajor-caju* grown on sawdust

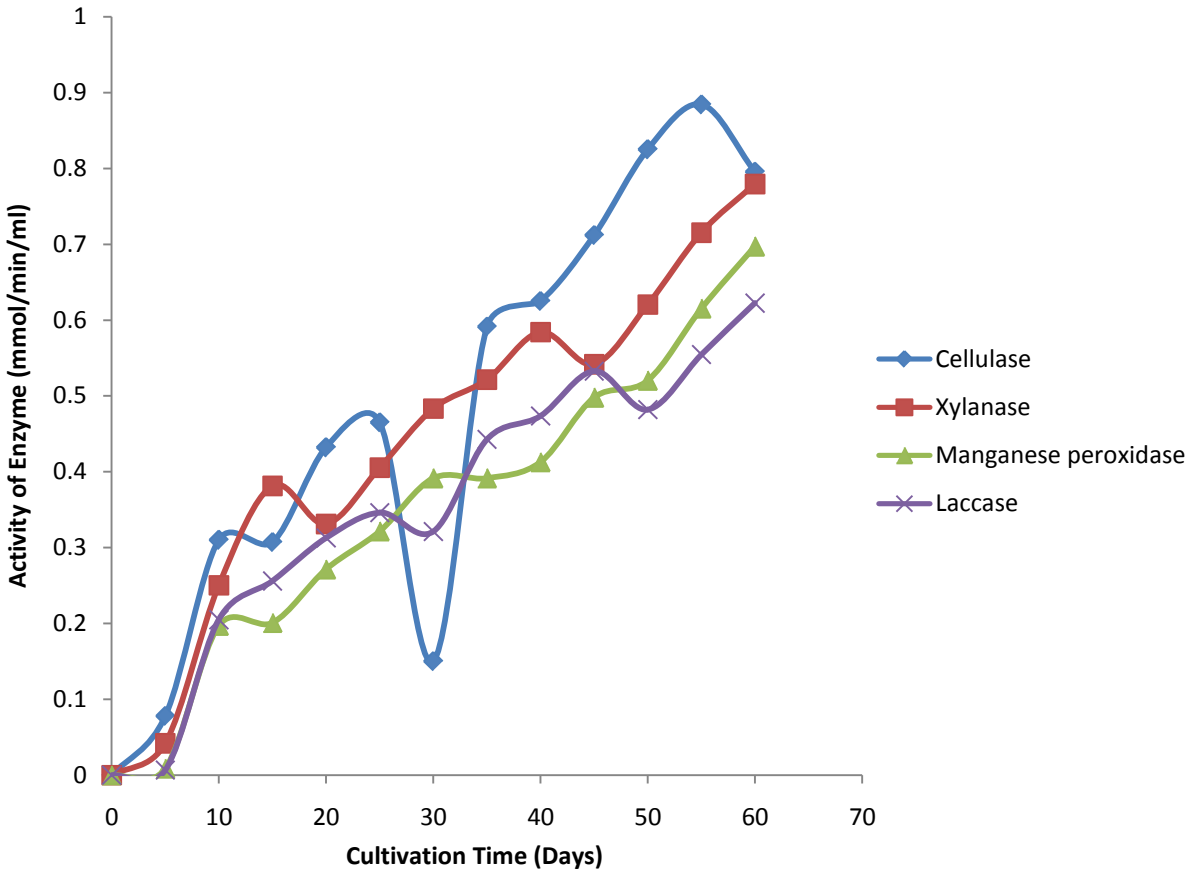


Figure 4. Time-course of activities of enzymes produced from *Pleurotus sajor-caju* grown on brewers spent grains

Table 3. Antinutrient composition of *Pleurotus sajor-caju* cultivated on different agro-industrial wastes

	Tannin (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)	Alkaloid (%)
Sawdust	6.72 ± 1.35a	162.48 ± 0.95b	4.82 ± 1.21ab	3.7 ± 0.99b
Rice Straw	5.46 ± 1.10b	149.79 ± 0.90a	4.24 ± 1.28a	2.11 ± 1.37a
Brewers Spent Grains	6.22 ± 1.32a	155.22 ± 1.13ab	4.88 ± 0.91ab	2.84 ± 1.21ab
Rice Bran	5.39 ± 1.18b	151.77 ± 1.08ab	4.35 ± 0.89b	2.36 ± 0.98a

3.3. Antinutrient Properties of *P. sajor-caju* Cultivated on the Agro-industrial Wastes

The antinutrient composition of edible mushrooms cultivated on different waste is shown in Table 3. Mushrooms grown on saw dust (SD) had the highest tannin content (6.72mg/100g), while the rice bran (RB) cultivated mushrooms had the least value (5.39mg/100g). Mushrooms grown on SD had the highest amount of phytate (162.48mg/100g) as well as alkaloids (3.7%). Rice straw (RS) mushrooms had the least amounts of phytate (149.794mg/100g), oxalate (4.24mg/100g) and alkaloid (2.11%). Brewers spent mushrooms (BS) had the highest amount of oxalate (4.88mg/100g).

Tannin content of sawdust and brewers spent grains were not significantly different each other but was significantly from tannin content of *P. sajor-caju* cultivated on rice straw and rice bran. Phytate content of *P. sajor-caju* cultivated on sawdust was significantly different from phytate content of *P. sajor-caju* cultivated on Rice straw and rice bran with no significant difference from *P. sajor-caju* cultivated on brewers spent grains. There is no significant difference in the oxalate content of *P. sajor-caju* cultivated on the four agro-industrial wastes. Alkaloid content of *P. sajor-caju* cultivated on sawdust was significantly different from *P. sajor-caju* cultivated on rice bran but had no significant difference from *P. sajor-caju* cultivated on rice straw and brewers spent grains.

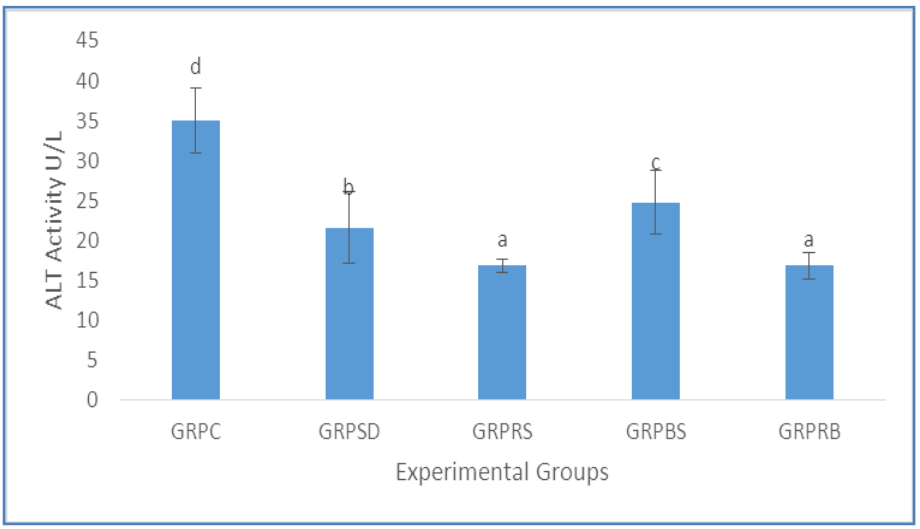
3.4. Toxicity Study on the Effect of *Pleurotus sajor-caju* on Some Liver Function Indicator. (Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Gamma Glutamyl Transferase (GGT) and Alkaline Phosphatase (ALP))

The effects of feeding of male albino rats with *Pleurotus sajor-caju* cultivated on different agro-industrial wastes revealed that the ALT enzyme activity (Figure 5) in male rats significantly decreased ($p \leq 0.05$) with the administration of the edible mushrooms compared to the control (GRPC) without edible mushroom administration. There was no significant difference in the ALT enzyme between the rice straw grown mushroom (GRPRS) and

rice bran grown mushroom administered groups (GRPRB) ($p \leq 0.05$), both of which had the least ALT activity. Among the edible mushroom groups, brewers spent grains produced mushrooms that had the highest significantly elevated activity of ALT ($p \leq 0.05$) on tested male albino rats.

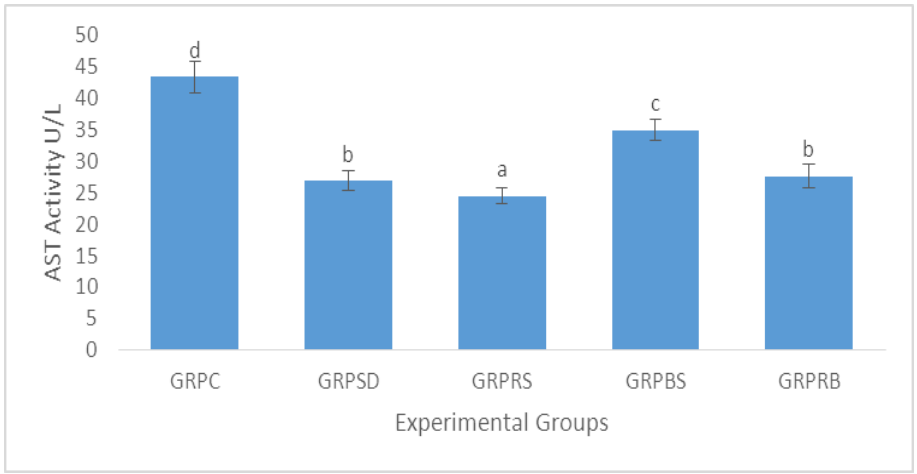
The effect of *P. sajor-caju* cultivated on the different substrates on AST is shown in Figure 6. The experimental groups manifested a significant decrease ($p \leq 0.05$) compared to the control (GRPC) without edible mushroom administration. Rice straw (GRPRS) cultivated mushroom significantly inhibited ($p \leq 0.05$) AST activity more than other mushrooms cultivated on other wastes. There was no significant difference in the inhibition level of AST between the mushrooms grown on rice bran (GRPRB) and saw dust (GRPSD) wastes ($p \leq 0.05$), but the two encouraged an activity of AST that was significantly higher than in the animals administered with mushrooms grown on rice straw (GRPRS) ($p \leq 0.05$). Group administered with brewer spent cultivated mushroom (GRPBS) had significantly high activity of AST ($p \leq 0.05$) compared to the other groups with other mushrooms cultivated on other wastes.

The effect of the edible mushroom cultivated on four different wastes saw dust (SD), rice straw (RS), brewers spent (BS) and rice bran (RB) was shown on the activity of gamma glutamyl transferase (GGT) in Figure 7. All the edible mushroom administered groups had GGT inhibiting activity, as a significant reduction in the enzyme activity was manifested in all the groups compared to the control group (GRPC) ($p \leq 0.05$). Animals in group administered with RB cultivated edible mushrooms had the least activity of GGT which was significantly reduced compared to GRPRS and GRPBS ($p \leq 0.05$), there was no significant difference compared to GRPSD ($p \leq 0.05$).



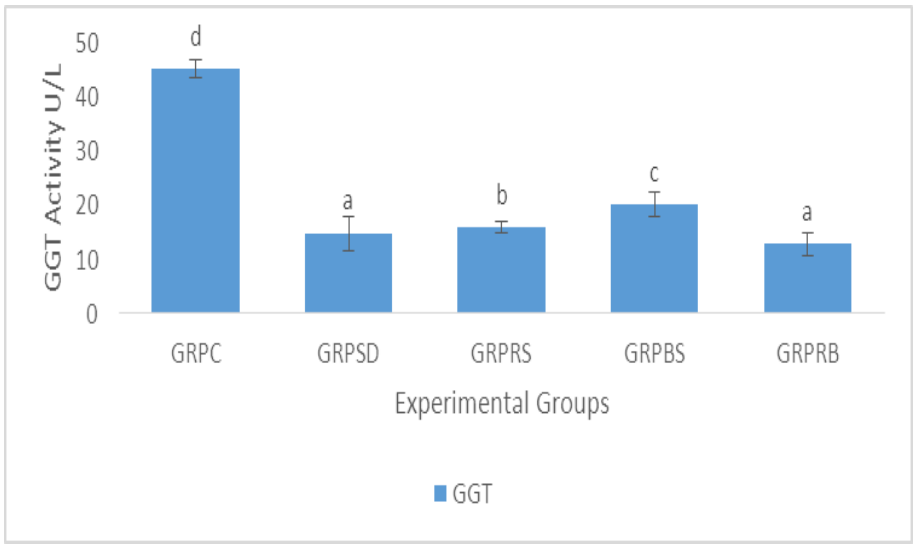
GRPC-control
GRPSD- Albino rats fed with mushrooms cultivated on Saw dust
GRPRS-Albino rats fed with mushrooms cultivated on Rice straw
GRPBS- Albino rats fed with mushrooms cultivated on Brewers spent grains
GRPRB- Albino rats fed with mushrooms cultivated on Rice bran

Figure 5. Effect of *Pleurotus sajor-caju* cultivated on four different wastes on the activity of Alanine amino transferase (ALT) in male rats



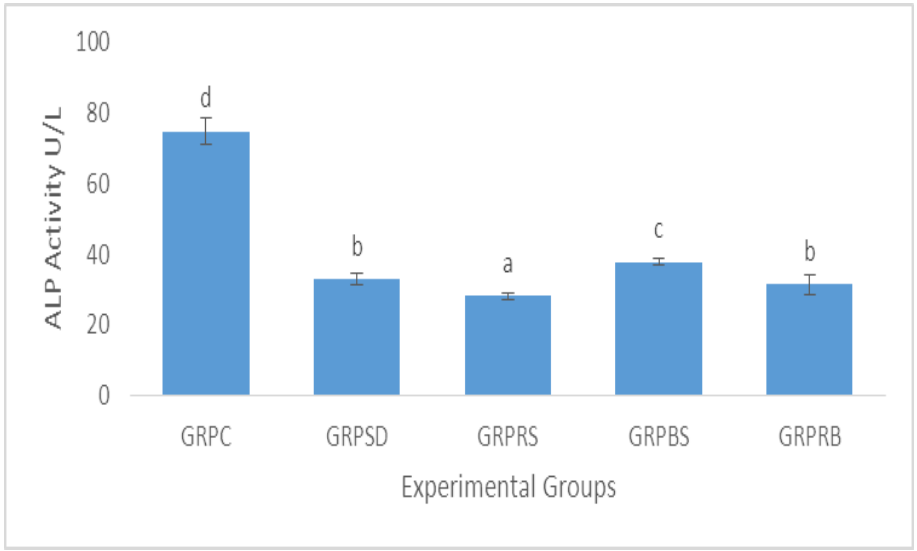
GRPC-control
GRPSD- Albino rats fed with mushrooms cultivated on Saw dust
GRPRS-Albino rats fed with mushrooms cultivated on Rice straw
GRPBS- Albino rats fed with mushrooms cultivated on Brewers spent grains
GRPRB- Albino rats fed with mushrooms cultivated on Rice bran

Figure 6. Effect of *Pleurotus sajor-caju* cultivated on four different wastes on the activity of aspartate amino transferase (AST) in male rats



GRPC-control
GRPSD- Albino rats fed with mushrooms cultivated on Saw dust
GRPRS-Albino rats fed with mushrooms cultivated on Rice straw
GRPBS- Albino rats fed with mushrooms cultivated on Brewers spent grains
GRPRB- Albino rats fed with mushrooms cultivated on Rice bran

Figure 7. Effect of *Pleurotus sajor-caju* cultivated on four different wastes on the activity of gamma glutamyl amino transferase (GGT) in male rats



GRPC-control
GRPSD- Albino rats that fed on mushrooms cultivated from Saw dust
GRPRS-Albino rats that fed on mushrooms cultivated from Rice straw
GRPBS- Albino rats that fed on mushrooms cultivated from Brewers spent grains
GRPRB- Albino rats that fed on mushrooms cultivated from Rice bran.

Figure 8. Effect of edible mushroom *Pleurotus sajor-caju* cultivated on four different wastes on the activity of alkaline phosphatase (ALP) in male rats

The effect of the edible mushroom cultivated on four different wastes saw dust (SD), rice straw (RS), brewers spent (BS) and rice brown (RB) was revealed on the activity of alkaline phosphatase (ALP) in Figure 8. The entire edible mushroom administered rat groups had ALP inhibiting activity, as a significant reduction in the enzyme activity was manifested in all the groups compared to the control group (GRPC) ($p \leq 0.05$). Animals in group administered with rice straw cultivated mushrooms had the least activity of ALP which was significantly reduced compared to GRPRB, GRPSD and GRPBS ($p \leq 0.05$), there was no significant difference between GRPRB and GRPSD ($p \leq 0.05$). GRPBS had the highest activity of ALP.

4. Discussion

The spawn run for the cultivation of *P. sajor- caju* on the four different agro industrial wastes varied from 24 to 32 days with pin head formation from day 25 to 45 days, while the fruiting body formation occurred from day 28 to 48 days depending on the substrate used. Results from this research are similar to the findings of Shah *et al.* [22] who stated that pinheads appeared 27 to 34 days after incubation at 17- 20°C. This result also agrees with Pathmashini *et al.* [23] whose fastest spawn run on *P. ostreatus* cultivated on sawdust was 21 ± 1day with pin head formation at 35 ± 1 day. However, this research does not agree with the findings of Buah *et al.* [24] who

reported that pin heads were formed at 12 days and that the average fruiting bodies were formed at 28 days of cultivating *Pleurotus* sp on grounded corn cob. Variations in spawn run rate may be attributed to the size of the substrate used. Smaller grains have greater number of inoculation points per kg than larger grains [25]. It was found that spawn run on smaller grains was higher than the larger grains. Also, different types of spawn may influence the productivity and growth of *P. sajor-caju* [23].

Time course of activities of enzyme produced from *Pleurotus sajor-caju* grown on rice straw (Figure 1) showed that the activity of most enzymes increased from the period of inoculation to formation of fruiting bodies. The lignocellulolytic enzyme activity is dependent on the composition of the substrate and on the carbon to nitrogen ratio as reported by Kahraman and Gurdal [26]. According to these authors, factors affecting the production of enzyme include availability of oxygen, the carbon and nitrogen concentration, the pH and the temperature. The result from this research is similar to the research of Jose *et al.* [27] who cultivated *Pleurotus ostreatus* on agro-industrial wastes and observed that the activity of lignocellulolytic enzymes increased throughout the period of cultivation. The results from this research showed that substrate composition and colonization time influenced the activity of lignocellulolytic enzymes.

The time-course of activities of enzymes produced from *Pleurotus sajor-caju* grown on rice bran showed an initial increase in enzyme activity (Day 0 - 15) followed by a decrease (day 15-35) in activity then a gradual increase in enzyme activity till the end of the cultivation period. Laccase and manganese peroxidase had the highest activity on rice bran (0.422mmol/min/ml;0.421mmol/min/ml), this result is similar to the findings of Chawachart *et al. [28]* who reported the highest laccase activity after 36 days of cultivation on rice bran (1.98U/g substrate) and that it was evident that laccase activity might increase if time course of enzyme production was considered [28].

The time-course of activities of enzymes produced from *P. sajor-caju* grown on sawdust showed that the activity of lignocellulolytic enzymes increased throughout the period of cultivation. Results in this study agree with the research work of Sherrif *et al.* [29] who reported a gradual increase in enzyme activity with incubation time on both rice straw and sawdust. Lignin degradation could be attributed to the ability of *Pleurotus* sp. to produce ligninases such as laccases and peroxidases in bulk [30].

The time-course of activities of enzymes produced from *Pleurotus sajor-caju* grown on brewers spent grains (Figure 4) revealed that cellulase and xylanase activity were higher than the activity of laccase and manganese peroxidase. Matsumoto [31] found that cellulase and xylanase activities increased during the development of fruiting bodies with the highest levels during mushroom maturation. This increase in the enzyme activities may be due to the fact that fungus needs to metabolize large amounts of carbon for mushroom maturation [32].

The antinutrient compositions of *Pleurotus sajor-caju* cultivated on the wastes was reported in Table 2. Aletor [33] reported that phytic acid in tropical specie ranged from 100 to 360mg/100g where samples were analyzed as a whole (without separating the cap and pileus). This report was in line with the findings of this research as the mushroom cultivated was analyzed as a whole and the results obtained from this study (149.79mg/100g – 162.48mg/100g) were found within this range. According to Akindahunsi and Oboh [34], phytate content of mushrooms was low when compared to green leafy vegetables whose phytate content was exceptionally high. Due to their strong chelating power, phytates act as a carrier or storage for trace metal minerals during plant growth [35]. Sandberg *et al.* [36] suggested that food processing such as cooking, fermentation, autoclaving and milling can reduce or eliminate the level of phytic acid by altering inositol hexaphosphate to other degradation forms, e.g penta-, tetra-, tri-, di- and monophosphate.

Tannins are known to retard growth through reduced digestion and absorption [37]. Condensed tannin in Table 2 varied from 5.39mg/100g to 6.72mg/100g. This research agrees with the findings of Aletor [33]; Akindahunsi and Oyetayo [38] who reported that tannin concentration in mushrooms were low (21mg/100g to 31mg/100g) when the cap, stalk and tuber of mushrooms were analyzed separately. These levels might not affect the nutritional potentials of mushroom parts since the results are all less than 10% of the total dry weight of the samples [39]. Study by Woldegiorgis *et al.* [40] also revealed the antinutrient compositions in edible mushrooms where phytate ranged from 31.3mg/100g to 242.8mg/ 100g and condensed tannin from 4.81mg/100g to 31.7mg/100g. Results from this research agree with the report of Woldegiorgis *et al.* [40].

Enzyme markers are liver function indicators of deterioration or inflammation in the liver. In the condition of liver damage, these enzyme markers increase in activities. The present study revealed that experimental feeding of albino rats with *Pleurotus sajor-caju* had no adverse effects on the enzyme indicators [Alanine Amino Tranferase (ALT), Aspartate Amino Transferase (AST), Gamma Glutamyl Transferase (GGT) and Alkaline Phophatase (ALP)] for liver function. These findings is similar to the report of Alam *et al.* [41] who noticed a decrease in the body weight of hypercholesterolemic rats fed with *P. ostreatus* and recorded that there was no adverse effects on enzyme profiles and some other parameters [41]. The dietary supplementation with the fruiting body powder provided natural plasma lipid and glucose lowering effects in experimental rats without adverse effects on the plasma biochemical parameters and liver function related enzyme activities [42]. The alterations in the activities of the enzymes can be ameliorated by pretreatment with nutraceuticals or pharmaceuticals with the tendency of ameliorating hepatopathy.

5. Conclusion

This research showed that *Pleurotus sajor-caju* produced variety of enzymes (Cellulase, manganese peroxidase, laccase and xylanase) on the agro-industrial wastes used (Sawdust, rice straw and brewers spent grains) and has no toxic effect on the liver. With the abundance of lignocellulosic wastes, favourable weather condition and adequate technical knowledge of mushroom production, private and government farms could be encouraged to go

into mass production of these species and other edible species. Enzymes play a significant role in mushroom development; however, *Pleurotus sajor-caju* which showed a higher production of manganese peroxidase and laccase can be used for the production of these enzymes. Cultivation of *P. sajor-caju* is therefore recommended for effective bioconversion of these agro-industrial wastes and the production of these enzymes, which could be of high advantage to research institutes and for industrial applications.

Conflict of Interests

The authors declare they have no conflict of interests.

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