CATALYTIC ACTIVITY AND PURIFICATION OF THERMOSTABLE LIGNASES FROM *GANODERMA* SP. ADE468 AND ITS POTENTIAL APPLICATION IN THE DECOLOURIZATION OF DYES

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Catalytic activity and purification of thermostable lignases from *Ganoderma* sp. ADEB468 and the potential of the enzyme in dye decolourization were investigated. The highest catalytic activity of laccase (Lac) (175.56 U/L), lignin peroxidase (LiP) (1313.98 U/L), and manganese peroxidase (MnP) (92.27 U/L) was supported by 0.1mM Na^{+}, 5.0 mM Fe^{3+}, and 1.0 mM benzoic acid. The optimum temperatures of 30°, 25°, and 30°C supported the highest catalytic activity. The enzymes were stable up to 70°C for the Lac and 90°C for the LiP and MnP. At 80°C and 90°C, 118.83 U/L and 74.55 U/L residual activities of the LiP and MnP were retained after 60 and 30 min of incubation. This implies that the *Ganoderma* sp. ADEB468 LiP and MnP were thermostable enzymes. The highest catalytic activity and stability of the Lac and LiP was supported by pH 6 and pH 3, respectively. The calculated Km (mM) and Vmax (U/L) for the Lac, LiP, and MnP were 0.391 and 2.5×10^{o}, 0.401 and 5.3×10^{-3}, and 0.118 and 2.0×10^{-4}, respectively. The purification step using ammonium sulphate precipitation, dialysis, and column chromatography (Sephadex – G100) was 6.25 fold and specific activity of 2.229 U/mg for the Lac, 4.97 and 1.946 U/mg for the LiP, and 7.47 and 1.343 U/mg for the MnP. The highest decolourization using crude lignases and a cell culture of *Ganoderma* sp. ADEB468 was 74.29% and 84.80% for Dylon green (26) 500 mg/L at 72 h and Dylon red (32)1000 mg/L at day 9. *Ganoderma* sp. ADEB468 under the studied conditions produced thermostable lignases that decolourized different dyes and, therefore, can be useful in the textile industry.

Keywords: Catalytic activity, lignases, purification, dye decolourization

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Introduction

Ganoderma species are polyporoid fungi of the order Polyporales (Hariharan and Nambisan, 2013). They produce numerous biological active compounds that have medicinal and pharmaceutical applications (Jo *et al*., 2011). Their ligninolytic enzyme system involves different types and characteristics with possibilities of biotechnological, industrial, and environmental applications (Stajic *et al*., 2010). Lignases, also known as ligninolytic enzymes, are biocatalysts that catalyze the breaking down or degradation of lignin model compounds. These enzymes are capable of forming radicals inside the lignin polymer, which results in destabilization of bonds and, finally, in the breakdown of the macromolecule of the lignin (Hofrichter *et al*., 1999).

Generally, white rot fungi are so important because they produce extracellular polyphenol oxidases, particularly lignin peroxidases, manganese peroxidases, and laccases, which are highly effective in degrading lignin (Revankar and Lele, 2006). Studies demonstrate that under certain conditions laccase and manganese peroxidases are able to oxidize both the phenolic and non-phenolic substrates (Cabaleiro *et al*., 2006).

These enzymes are characterized by broad substrate specificity, the ability to form reactive radicals, and have strong oxidative mechanisms which enable them to degrade a wide variety of pollutants such as textile and pulp mill effluents, organochloride agrochemicals, and other synthetic aromatic compounds (Nyanhongo *et al*., 2002). One of the limitations of these enzymes for biotechnological use is the lack of capacity to produce them in a high quantity (Patrick *et al*., 2010).

Ligninolytic enzymes also play an important role in various processes such as biopulping, biobleaching, textile dye decolourization, and the treatment of agricultural residues and industrial waste waters (Moldes *et al*., 2007; Rodriguez-Couto *et al*., 2006; Rosales *et al*., 2007). Chemical and physical degradations are very expensive and involve inefficient processes; therefore, biological degradation which presents an alternative treatment for economic and ecological reasons is more acceptable and feasible (Croan, 2000; Ćilerdžić *et al*., 2011). Lignases have also been involved in the conversion of plant wastes to low molecular weight compounds with high nutrition valued food (mushroom fruiting bodies) and feeds (Ćilerdžić *et al*., 2011). Dyes are widely used within the food, pharmaceutical, cosmetic, textile, and leather industries. Part of the dyes is released into the water during industrial processing which results in highly coloured wastewaters that affect the aesthetics, water transparency, and gas solubility in water bodies (Raghunathan, 2011). There is also a general concern about the toxicity of some of these dyes; thus, appropriate treatment technologies are required (O'Neill *et al*., 1999). Biotechnological approaches were proved to be potentially effective in the treatment of this pollution source in an eco-efficient manner (Borchert and Libra, 2001). Therefore, this research aimed at the optimization of the cultural condition, purification, and characterization of thermostable lignases from *Ganoderma* sp. and its potential application for dye decolourization.

Materials and Methods

Microorganism

Ganoderma sp. ADEB468 was obtained from the culture collection of our previous experiments in the Department of Microbiology, Faculty of Science, University of Ibadan, Southwest Nigeria. Stock cultures were maintained on malt extract agar slants at 4°C.

Production of Lignases Using Submerged Cultivation

Production of lignases by *Ganoderma* sp. ADEB468 was done using Kirk medium (Tien and Kirk, 1983) containing per 1000 ml: 10.0 g - glucose, 0.2g - ammonium tartrate, 3.28 g - sodium acetate, 2.0 mg - thiamine, 2.0 g - H_2KPO_4, 0.53 g - $MgSO_4.7H_2O$, 0.1 g - $CaCl_2$, 1 mg - $CuSO_4$, 5 mg - $MnSO_4$, 0.1 mg - H_3BO_3, 0.1 mg - $NaMoO_4.2H_2O$, 1.0 mg - $ZnSO_4.7H_2O$, 1.0 mg - $CoCl_2$, 10 mg - NaCl, 1.0 mg - $FeSO_4.7H_2O$, and 0.1 mg - $AlK(SO_4)2.12H_2O$. The medium was sterilized

and inoculated with 2 agar plugs of growing culture of Ganoderma sp. ADEB468. The inoculated medium was incubated on a shaker at 30°C for 21 days. After incubation, the cultivation broth was filtered and the filtrate was analyzed for lignases activity.

Purification of Lignases

The crude enzyme was purified from culture broth using 2 $(NH4)_2SO_4$ precipitation steps. The protein precipitate was dissolved in a 50 mM (pH 5.0) citrate phosphate buffer (Ding *et al*., 2012). The pooled protein precipitate from the first and second steps was dialyzed in the same buffer solution to remove $(NH4)_2SO_4$ (Ding *et al*., 2012). The dialysate was futher purified using column chromatography.

The desalted enzyme solution (dialysate) was applied to a Sepharose gel G-100 (Sigma-Aldrich Corp., St. Louis, MO, USA) Fast Flow column pre-equilibrated with a pH 5.0 citrate phosphate buffer solution. The column was washed with the buffer and the absorbed proteins were eluted using 0.2 mol/L NaCl in a citrate phosphate buffer at a flow rate of 0.5 mL/min (Ding *et al*., 2012).

Determination of the Effect of Purification on the Lignases Activity and Protein Content

The lignases' activities and the total protein content of the enzymes were determined at each stage of purification (i.e. the crude, ammonium sulphate precipitated, dialysed, and the pooled column purified enzymes) to determine the effect of purification of the enzymes.

Characterization of Lignases from *Ganoderma* sp. ADEB468

Effect of Metallic Ions, Temperature, pH, Substrate and Enzyme Concentration on the Lignases' Catalytic Activity and Stability

The effect of the metallic ions, temperature (10 - 60°C), pH (3.0 – 9.0), substrate concentration 2, 2'- azinobis-(3-ethylbenzthiazoline-6-sulfonate (ABTS) (0.1 - 1.0mM), Veratryl alcohol (1 - 10 mM), and $MnSO_4$ (0.1 - 1.0 mM)), and enzyme concentration on the lignases' catalytic activity and stability was investigated. The residual Lac activity was determined using ABTS (1mM) as the substrate and the absorbance was measured at 420 nm (ε_{420} = 36000 $M^{-1}cm^{-1}$). One enzymatic activity was defined as the quantity of enzyme that produced 1 µmol of oxidized product.

For the LiP, veratryl alcohol (10 mM) was used as the substrate and the absorbance was immediately measured in 1-min intervals after the addition of H_2O_2. One unit (U) of LiP activity was defined as the activity of an enzyme that catalyzes the conversion of 1 µmole of veratryl alcohol per minute.

For the MnP, $MnSO_4$ (0.1 mM) was used as the substrate and the absorbance was measured at 610 nm (ε_{610} = 22000 $M^{-1}cm^{-1}$). The method is based on the oxidation of Mn(II) to Mn(III). One enzymatic activity was defined as the quantity of enzyme that produced 1 µmole of oxidized product (Pol *et al*., 2012; Martos *et al*., 2013)

Determination of the Kinetic Properties of *Ganoderma* sp. ADEB468 Lignases

The kinetic properties of the purified lignases produced by *Ganoderma* sp. were determined using the Michaelis-Menten constant (Km) and catalytic constants (Kcat) and the specific activity values were determined using a Lineweaver-Burk plot.

Application of Lignases in Dye Decolourization

The decolourization potential of the lignases from *Ganoderma* sp. ADEB468 was investigated by using the crude enzyme and cell culture at different concentrations for decolourization of different dye concentrations (0.5 - 1.0 g). Dylon dye (Spotless Punch Ltd., Redhill, UK) was used based on its availability and high quality. Decolourization was determined by measuring the absorbance of the decolourization medium at different wavelengths depending on the dye (Dylon Blue (18) at 405 nm, Dylon Green (26) at 570 nm, and Dylon Red (32) at 660 nm) (Kumar *et al*., 2011).

Table 1. Effect of different concentrations (mM) of metallic ions on catalytic activity of purified lignases produced by *Ganoderma* sp.

		DIFFERENT CONCENTRATIONS (mM) OF METALLIC IONS										
Lignases	**Conc.**	**EDTA**	**Benzoic acid**	**Dinitrophenol**	**MeCl**	**LiCl**	**KCl**	**FeCl**	**BaCl**	**NaCl**	**CaCl**	**Control**
Lac	0.1	30.11	63.33	156.11	125.00	147.22	136.11	132.78	116.67	175.56	122.78	120
	0.5	151.67	142.78	153.89	156.11	142.78	145.56	157.22	118.89	141.67	146.67	
	1.0	145.00	141.11	153.89	157.78	142.22	112.22	167.78	164.44	91.11	155.56	
	5.0	173.33	143.89	147.78	144.44	145.00	158.89	128.33	142.78	153.89	141.67	
LiP	0.1	30.11	43.01	258.07	10.75	38.71	25.81	165.59	47.31	10.75	49.46	83.87
	0.5	40.86	25.81	8.60	30.11	62.37	356.99	45.16	40.86	49.46	53.76	
	1.0	43.01	21.51	86.02	38.71	38.71	30.11	440.86	62.37	30.11	47.31	
	5.0	73.12	79.57	40.86	45.16	4.30	62.37	1313.98	47.31	51.61	6.45	
MnP	0.1	84.09	78.64	51.82	70.00	55.00	61.36	48.64	37.27	64.55	79.09	58.12
	0.5	80.91	83.18	85.91	66.36	73.64	81.82	39.09	55.00	83.18	68.64	
	1.0	89.09	92.27	77.73	74.09	65.46	71.36	68.64	65.00	61.36	50.46	
	5.0	81.82	83.64	83.64	3.18	8.55	78.64	76.36	16.82	57.27	79.09	

The percentage decolourization was calculated, as follows:

$$\frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100\%$$

Results and Discussion

Effects of Metallic Ions and Inhibitors on the Lignases' Catalytic Activity

Different concentrations of metallic ions had significant effects on the lignase catalytic activity (Table 1). For the Lac, 30% of the metallic ion at a higher concentration (5.0 mM) had an inducible effect on the activity. NaCl 0.1 mM had a strong inducible effect on the Lac activity, while ethylenediaminetetraacetic acid (EDTA) 0.1mM repressed the activity of the Lac (175.56[a] U/L). Fifty percent of the metallic ion at a high concentration induced the optimum LiP activity. The highest activity (1313.98 U/L) was induced by FeCl2 5.0 mM while CaCl2 5.0 mM repressed the LiP activity (6.45 U/L). Eighty percent of the metallic ion at 0.1 – 1.0 mM concentrations had a strong inducive effect on MnP activity. Benzoic acid 1.0 mM induced the highest activity (92.27 U/L) of the MnP, while mercury chloride 5.0mM had a strong repressive effect on the activity of the MnP. Ligands do not require any specific metallic ion for catalytic activity but a strong repressive effect was observed at higher concentrations (5.0 Mm) of mercury chloride and calcium chloride.

The lignases retained their activity in the presence of the different metal ions used in this study. This agrees with the reports of Sadhasivam *et al*. (2008), that metal ions at a concentration of 1 mM had no significant effect over *Trichoderma harzianum* WL1 laccase activity, and Ahammed (2002), who reported that Fe^{3+} had no inhibitory effect on the lignin peroxidase activity of *Aspergillus* sp. SIP 11. The response of the enzymes to metal ions also depends on the fungal species (Hassan *et al*., 2012). The inhibitors used in this study are discovered not to have a significant effect on lignases production. Atalla *et al*., (2013) reported that EDTA has a low effect on the laccase activity of *Trematosphaeria mangrovei*. The result in this study is not in agreement with the work of Ahammed (2002), who reported the inactivation of lignin peroxidase produced by *Aspergillus* sp. SIP 11 by EDTA, and the work of Asgher and Iqbal (2011) who reported that EDTA had a partial inhibition on the activity of the purified MnP of Trametes versicolor IBL-04.

Effect of the Temperature on the Lignases Catalytic Activity and Stability of *Ganoderma* sp. ADEB468 Lignases

The effect of the temperature on the catalytic activity and stability of *Ganoderma* sp. ADEB468 lignase was investigated Figure 1(a) and 1(c)). The highest Lac activity was at 30°C. The enzyme was stable up to 70°C and reduced at 80°C and 90°C. *Ganoderma* sp. ADEB468 Lac was stable at 25-70°C at 10-60 min incubation. There was a loss in activity at 10, 15, and 60 min after incubation at 90°C.

The highest temperature was 25°C for the LiP activity and the stability was up to 90°C with the highest recorded at 80°C for 10 min. There was no loss in LiP activity over 60 min at 25-90°C. At 80°C, 195.70 U/l of the residual LiP activity remained after 10min, while about 118.83 U/L of residual activity was retained after 60min of incubation, which implies that *Ganoderma* sp. ADEB468 LiP is a thermostable enzyme. About 83.87 U/L of LiP activity was retained after 10 min when incubated at 90°C. The highest activity was recorded at 80°C (195.70 U/L) after 10 min of incubation.

The MnP had the highest catalytic activity at 30°C and stability was up to 90°C at 60 min with the highest recorded at 90°C for 30 min. There was no loss in *Ganoderma* sp. ADEB468 MnP enzyme activity when incubated at 60 min. About 40.91U/L of residual activity was recorded after incubation at 90°C for 60 min. The highest residual activity (74.55U/L) was recorded at 90°C on incubation for 30 min. This implies that *Ganoderma* sp. ADEB468 MnP was a thermostable enzyme.

In this study, the optimum temperature for the Lac activity was 30°C. Enzymes with optimum temperatures below 35°C have been reported; for example, Ko *et al*. (2001) reported

that Lac from *Ganoderma lucidum* had its highest activity at 25°C. The Lac remained stable up to 70°C for 1 h and a sharp decline was observed above this temperature. This is in agreement with the work of Raghunathan (2011) who reported that the Lac of *Trametes* sp remained stable at 70°C for more than 1h. The optimum temperature for LiP activity was 25°C. This result agrees with the work of Patel *et al*. (2007a) who reported a similar optimum temperature for the LiP of *Abortiporus biennis* MTCC-1176, *Pestalotia bicolor* MTCC-372, *Heterobasidion annosum* MTCC-146, and Glaephyllum striatum MTCC-1117. The enzyme remained stable up to 90°C with the highest stability at 80°C for 10 min. This is not in agreement with most of the work on the LiP optimum temperature for activity. The MnP optimum temperature was 30°C. This is in agreement with the report of Praveen *et al*. (2012) who reported an optimum temperature range of 25-40°C for manganese peroxidase of *Aspergillus terreus, Phlebia floridensis, Schizophyllum* F17, and *Phanerochaete chrysosporium*. The enzyme retained its stability up to 90°C which was contrary to

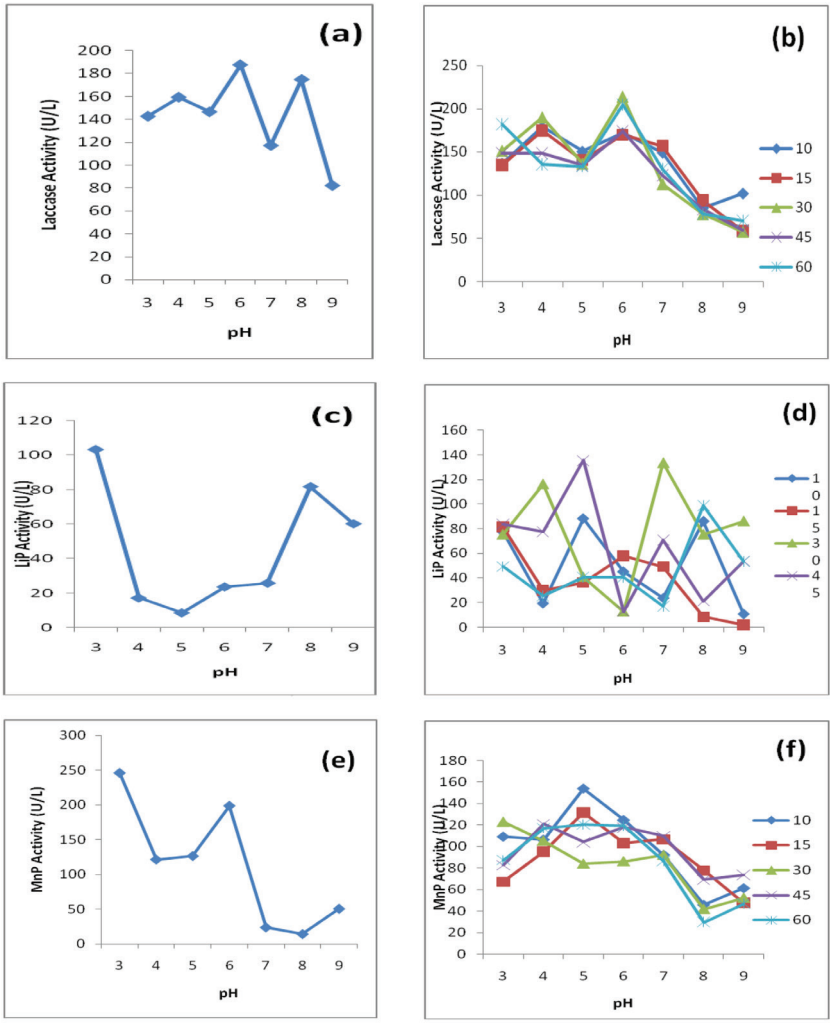


Figure 1. The effect of pH on Lac/LiP/MnP activity and stability

most studies, for example, *Phanerochaete chrysosporium* lost its activity completely after 15 min at 60°C (Rodriguez-Couto *et al*., 2006), and *Steruem ostrea* was totally inactivated at 65°C (Praveen *et al*., 2012). The differences in thermal stability at different temperatures may depend on the different sources of the enzymes. This has, however, no connection with the optimum growth of the fungi. The temperature stability varies considerably. The high thermostability is a desirable feature of an enzyme for various industrial applications (Bermek *et al*., 2004).

The Effect of the pH on the Lignases Activity and Stability

The effect of the pH on enzyme activity was determined, as shown in Figure 2(a-c). It was observed that the highest Lac activity and stability was recorded at pH 6. The Lac activity was stable between pH 3 and 7 but reduced at pH 8 and 9. At pH8 and 9 about 29.55 and 46.82 U/L was retained after incubation for 60 min. The highest activity was recorded at pH 5 at 10 min after incubation at 10°C.

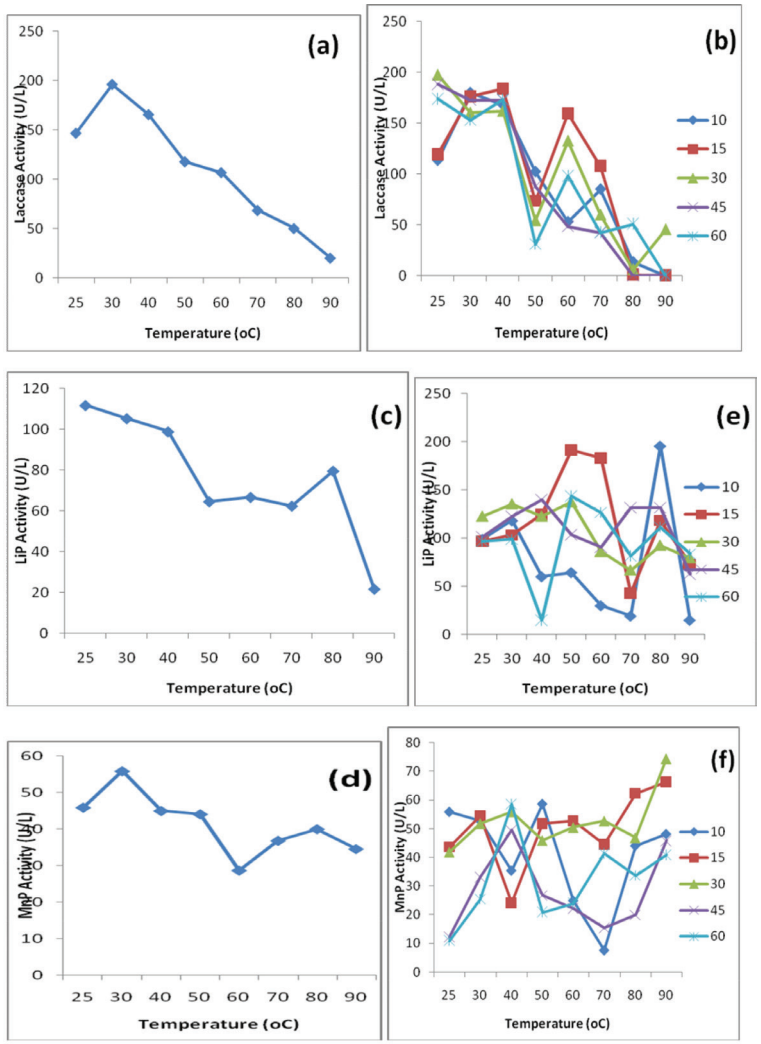


Figure 2. The effect of temperature on Lac/LiP/MnP activity and stability

The LiP had the highest activity at pH 6 and stability between pH 3 and 9 with the highest stability at pH 5. At pH 5, *Ganoderma* sp. ADEB468 Lac retained more than 135.48 U/L activity after 45 min. At pH 8, less than 100 or 98.93 U/L of the LiP activity remained after 60 min of incubation. *Ganoderma* sp. ADEB468 had the highest residual activity (98. 93 U/L) at pH 8. The least activity was recorded at pH 7 (17.20 U/L) after incubation for 60 min.

The MnP had the highest activity at pH 3 and stability at pH 5 (135.48 U/L) after 45 min of incubation. The pH remained stable between pH 3 and 7 but reduced at pH 8 and 9. The MnP was stable at pH 4 and pH 6 and had the highest activity (119.09 U/L) at pH 5 after 60 min of incubation and the lowest residual activity at pH 8 and pH 9 (29.55 U/L) after 60 min of incubation.

The highest pH for catalytic activity of the Lac was 6.0. This is in agreement with the work of Bulter *et al*. (2003) who discovered that the optimum pH for *Myceliophtora thermophila* Lcc 1 using ABTS as the substrate to be 6.0. This report is not in agreement with the work of Ding *et al*. (2012) who reported the optimum pH for the laccase activity of *Ganoderma lucidium* to be 3.0. The enzyme was stable up to pH 9 but a decreased activity was observed from pH 7. This result is in agreement with the reports of Kiiskinen *et al*. (2002), Palmieri *et al*. (2003), and Ben Younes *et al*. (2007) who reported neutral to alkaline pH for laccase stability for *Melanocarpus albomyces, Pleurotus ostreatus,* and *Perenniporia tephropora*, respectively. The optimum pH for lignin peroxidase in this study was 3.0. This agrees with the work of Patel *et al*., (2007b) who reported that the optimum pH for *Phanerochaete chrysosporium* ATCC-24725 lignin peroxidase activity was 3.0. The lignin peroxidase in this study retained its stability up to 9.0 with the highest stability at pH 5.0. The optimum pH for manganese peroxidase activity was 3.0. The enzyme remained stable at pH 9.0 with the highest stability at pH 5.0 and a reduced stability at above pH 7.0. This is in agreement with the work of Praveen *et al*. (2012) who reported high pH stability for the manganese peroxidase of *Stereum ostrea*.

The optimum pH values, for which the enzyme activity is the optimum, are characteristic for each enzyme. In this pH optimum domain the proton acceptor and proton donor groups of the active centre are in an ionized state necessary for the enzyme to be active. Outside this domain the binding of the substrate is not possible and, if the pH value exceeds a certain limit value, the enzyme can be irreversibly denaturized. This pH optimum depends on the environment composition, temperature, and enzyme stability in an acid and alkaline environment. The pH stability domain does not necessarily coincide with the reaction rate optimum domain (Xu, 1997).

Effect of the Enzyme Concentrations on the Enzyme Activity.

The effect of the enzyme concentrations on the enzyme activity is shown in Figure 3 (a-c). The Lac had the highest activity at a concentration of 80 µl. For An increase in the enzyme concentration from 50 µl - 200 µl leads to a reduction in the catalytic activity of the enzyme. This may be as a result of the saturation of the catalytic site and feedback inhibition from the product. A futher increase in the enzyme concentration from 200-300 µl leads to a futher increase in the catalytic activity of the enzyme. This may be due to the availability of the active site for futher cataysis.

Effect of the Substrate Concentration on Enzyme Activity.

Figure 4(a-c) shows the effect of the substrate concentrations on the Lac and LiP activity. The Lac and LiP activity increased with the increase in the substrate concentration up to 0.7 mM. The ABTS of 1.0 mM and 0.7 mM of veratryl alcohol were the highest for the Lac and LiP activity. The MnP activity increases with an increase in the substrate concentration up to 0.9 mM and the activity reduced at 1.0 mM $MnSO_4$ (Figure 5(c)).

The Kinetic Properties of *Ganoderma* sp. ADEB468 Lignases Enzyme

The kinetic properties of the purified

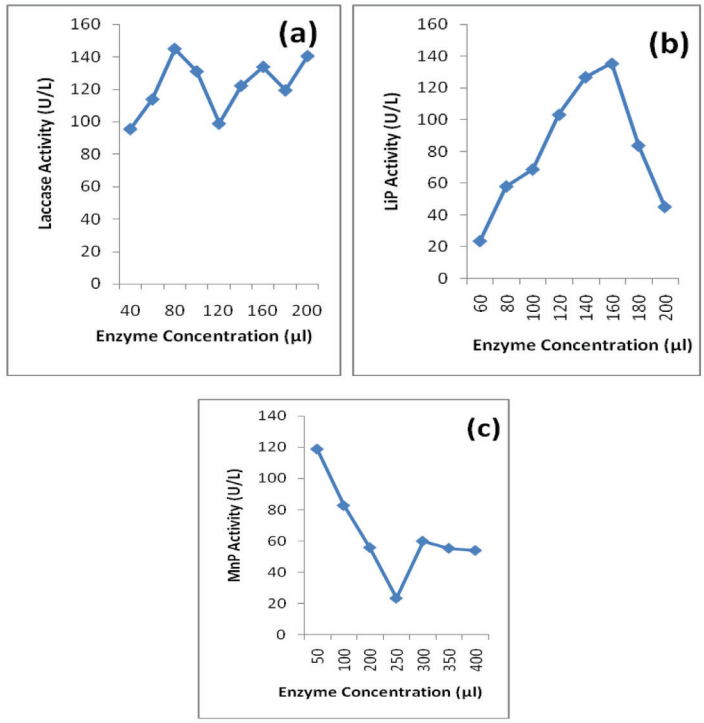


Figure 3. Effect of enzyme concentrations on Lac (a), LiP (b), and MnP (c) activity

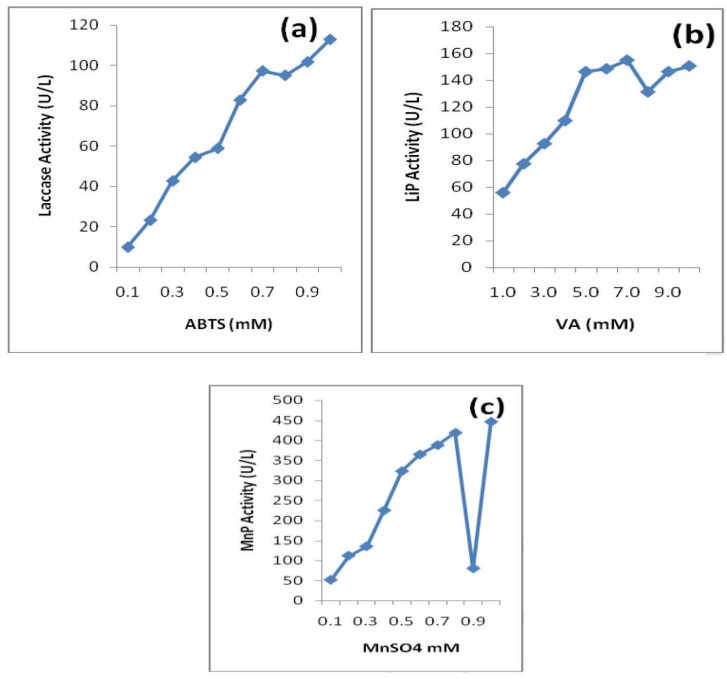


Figure 4. Effect of substrate concentrations on Lac (a), LiP (b), and MnP (c) activity

lignases were determined using various concentrations of ABTS as the substrates and the Michealis constant Km and Vmax.

The Lineweaver-Burk plots for the Lac, LiP, and MnP are shown in Figure 5(a-c).

The calculated Km (mM) and Vmax (U/L) for the Lac, LiP, and MnP were 0.391 and 2.5 × 10-3, 0.401 and 5.3 × 10^{-3}, and 0.118 and 2.0 × 10-4, respectively. Enzyme concentrations of 80 µl, 160 µl, and 50 µl supported the highest activity of the 3 enzymes under study. The calculated Km and Vmax for the Lac, LiP, and MnP are shown in Table 2.

The lignases' activity was greatly affected by different concentrations of the substrate and enzyme. The reaction was observed to increase gradually with an increase in the substrate concentration until it reached the maximum and then declined. An increase in the concentration of the substrate enhanced the lignases' activity. This result obeys the Michaelis - Menten equation (Dixon and Webb, 1971) from which it is reported that, for all the enzyme processes, the rate of reaction depends upon the concentration of both the enzyme and its substrate, other conditions being constant. The Km of an enzyme depends on the substrate, the pH of the reaction, and the culture condition (Banerjee and Vohra, 1991). For the Lac, the Km obtained in this study is 0.391mM with ABTS at pH 5.0 and the temperature at 30°C. Niladevi, (2008) reported a similar Km for *Streptomyces psammoticus using* ABTS as the substrate. Fukushima and Kirk, (1995) reported that the *Ceriporiopsis subvermispora* Km at pH 3.5 using guaiacol was 1.6 mM. The LiP Km in this study was 0.401 mM with veratryl alcohol at pH 5.0 and temperature 30°C. Ollikka *et al*. (1993) reported that the Km for *Phanerochaete chrysosporium* F-1767 using veratryl alcohol was 0.33 mM and Yadav *et al*. (2012) reported the Km value of *Lenzitus betulina* MTCC1183 using veratryl alcohol to be 54µM. The Km value for the MnP in this study is 0.118mM using $MnSO_4$ at pH 5.0 and a temperature of 30°C. Bermek *et al*. (2004) reported that the Km for *Trichophyton*

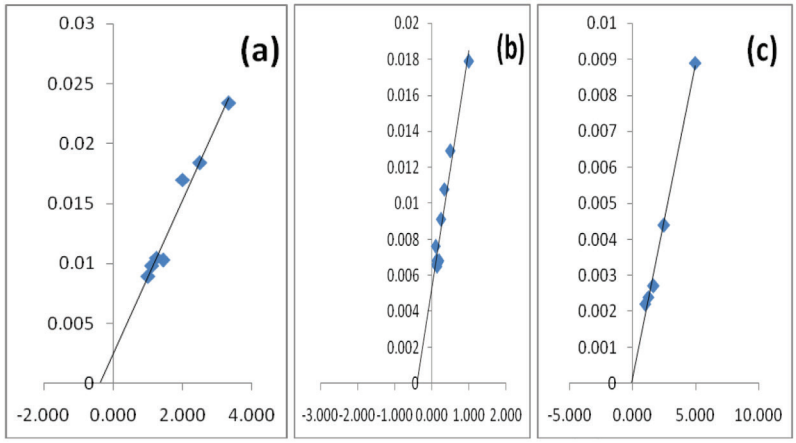


Figure 5. Lineweaver-Burk plots for Lac (a), LiP (b), and MnP (c) Km and Vmax

Table 2. Km and Vmax of purified Lac, LiP, and MnP of *Ganoderma* sp. ADEB468

Enzyme	Km (mM)	Vmax (U/L)
Laccase	0.391	2.5 x 10-3
Lignin Peroxidase	0.401	5.3 x 10-3
Manganese Peroxidase	0.118	2.0 x 10-4

rubrum LSK-27 using Mn^{2+} was 0.063 mM. Also, Praveen *et al*. (2012) reported the Km value for manganese peroxidase of *Stereum ostrea* using Mn^{2+} to be 50 µM.

Enzyme Assay of the Fractions

The Lac, LiP, and MnP activities of the 49 fractions collected from the column chromatography were determined. It was observed that the highest peaks for the Lac were at fractions 5, 7, 9, and 46. For the LiP they were at fractions 2, 14, 33, and 49. The MnP highest peaks were at fractions 4, 6, 8, and 16, as shown in Figure 6(a-c).

Purification of Lignases from *Ganoderma* sp. ADEB468

Table 3 shows the purification of lignases from *Ganoderma* sp. ADEB468. The purification step using ammonium sulphate, dialysis, and

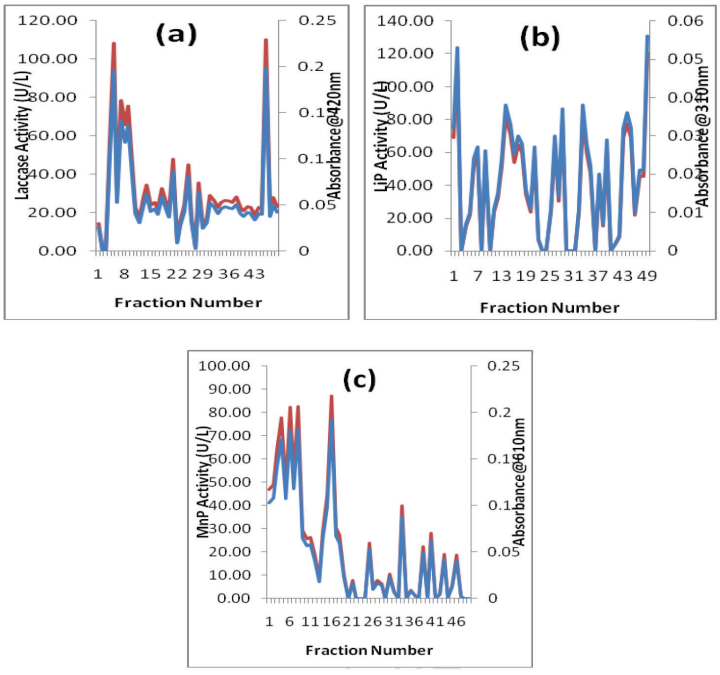


Figure 6. Fractional assay of Lac/LiP/MnP activity

Table 3. Purification of lignases from *Ganoderma* sp. ADEB468

Step	Purification steps of lignases												
	Activity (U/mL)				Specific Activity(U/mg)			Fold			Purification Yield (%)		
	Protein (mg/ml)	Lac	LiP	MnP	Lac	LiP	MnP	Lac	LiP	MnP	Lac	LiP	MnP
Crude	0.345	0.123	0.135	0.062	0.357	0.391	0.180	1.00	1.00	1.00	100	100	100
$(NH_4)_2SO_4$ Precipitated	0.164	0.218	0.157	0.142	1.329	0.957	0.866	3.73	2.45	4.82	94.74	94.74	94.74
Dialyzed	0.108	0.173	0.196	0.13	1.602	1.815	1.204	4.49	4.64	6.70	89.12	89.12	89.12
Sephadex-G100	0.066(Lac)												
	0.061(LiP)												
	0.089(MnP)	0.093	0.1	0.083	2.229	1.946	1.343	6.25	4.97	7.47			

Note: Activity: $U/Ml_{\text{Specific activity: U/mg}}$

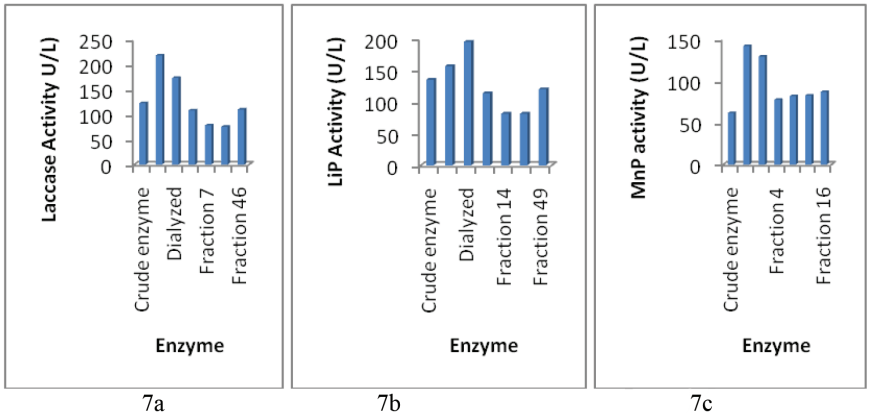
column chromatography (Sephadex – G100) was 6.25 fold and specific activity 2.229 U/mg for the Lac, 4.97 fold and specific activity 1.946 U/mg for the LiP, and 7.47 fold and specific activity 1.343 U/mg for the MnP.

Lignases produced by *Ganoderma* sp. ADEB468 were purified. There was an increase in the specific activities and purification fold for the 3 enzymes. This is in agreement with the works of Ding *et al*. (2012), Yadav *et al*. (2010) and Praveen *et al*. (2012) who reported increased specific activities for Lac, LiP, and MnP, respectively. This increase in specific activity indicates enzyme efficiency and purity. Assay of crude, purified and different fractions of lignases from *Garnoderma* sp. ADEB468 is shown in Figure 7(a-c).

Potential Application of *Ganoderma* sp. ADEB468 Lignases in the Decolourization of Dyes

The potential application of *Ganoderma* sp. ADEB468 lignases in the decolourization of dyes was investigated. The decolourization using enzyme and cell culture of *Ganoderma* sp. ADEB468 is shown in Figure 8(a-b). The decolourization using crude lignases and the cell culture of *Ganoderma* sp. ADEB468 ranged from 5.96-74.29% and 22.88- 84.80% with the highest percentage decolourization recorded in Dylon green (26) 500 mg/L at 72 h and Dylon red (32) 1,000 mg/L at day 9. It was observed that the higher the concentration of the dye, the lower the percentage decolourization and the longer the time, the higher the percentage decolourization. However, there were a few exceptions to these. The best decolourized dye when crude lignases were used was green, followed in order by blue and red, while it was red, followed in order by blue and green when cell culture was used.

In this study, the highest decolourization was observed in Dylon green (26) at 500 mg/L at 72 h. Zouari-Mechichi *et al*. (2006) reported similar results on the decolourization of some textile dyes by the crude enzyme of *Trametes trogii*. When cell culture was used, the highest decolourization was observed in Dylon red (32) at 1,000 mg/L at day 9. Raghunathan (2011) reported similar results in the decolourization of acid orange-7 by *Phanerochaete chrysosporium*. Generally, except for a few exceptions, there was a decrease in the percentage decolourization with an increase in dye concentrations which is in agreement with the report of Renganathan *et al*. (2006). High dye concentrations implied fewer average attacks of lignases to each dye molecule and hence a slower colour removal rate (Young and Yu, 1997). The reduction could be as a result of either enzyme inhibition by some products generated in the decolourization process or substrate inhibition (Osma *et al*., 2007) and also to the different redox potential of lignases due to the microbial source (Li *et al*., 1999). In addition, the reduction using cell culture could also be due to repression of fungal



7a 7b 7c

Figure 7. Assay of crude, purified, and different fractions of lignases from *Ganoderma* sp. ADEB468

growth as the dye concentration increases (Paszczynski *et al*., 1992). Structural differences in the dyes may also cause varying decolourization rates (Paszczynski *et al*., 1992). Each dye molecule contained a chromophore and its colour disappeared only after the chromophore structure was destroyed, which might need many attacks of the lignases radicals (Ahammed, 2002).

Conclusions

Ganoderma sp. ADEB468 under the studied conditions can produce Lac, LiP, and MnP. The highest lignases catalytic activity was at an acidic pH and temperature of 25° and 30°C. Moreover, the stability was maintained at a pH of 3-7 and a temperature of 25-90°C. This implies that it can be used at wider ranges of the pH and temperature. The study further confirmed the thermostability of the lignases produced by *Ganoderma* sp. ADEB468. *Ganoderma* sp. ADEB468 under the studied conditions produced lignases that decolourized different dyes and, therefore, can be useful in the textile industries.

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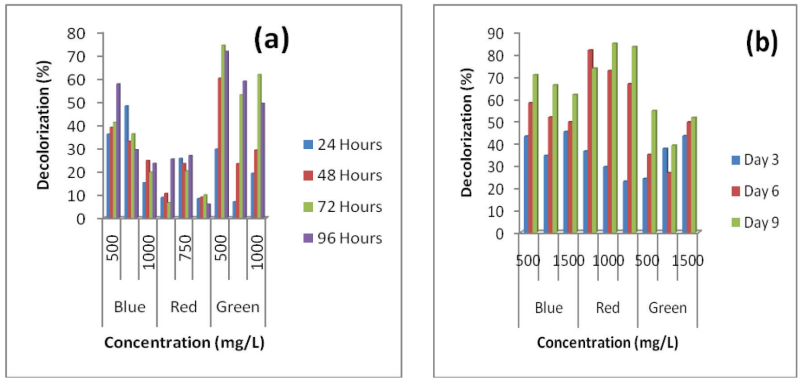

Figure 8. Dye decolourization of crude enzyme (a) and cell culture (b) of Ganoderma sp. ADEB468

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