EFFECT OF ANNONA SENEGALENSIS EXTRACT ON THE GROWTH AND BIOACTIVITY OF *GANODERMA LUCIDUM.*

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ABSTRACT

Ganoderma lucidum is a medicinal mushroom that can be used in conjunction with other medicinal plants such as *Annona senegalensis* in the treatment of various disease conditions. This study was designed to determine the antimicrobial and antioxidant properties of polysaccharide of *G. lucidum* grown in medium containing extract of *A. senegalensis*. *Ganoderma lucidum* grown in a medium containing extract of *A. senegalensis* was assessed for its antimicrobial activity using agar-well and disc diffusion methods. Antioxidant capacity of the cultivated mushroom was determined using established protocols. A higher growth rate (2.14 g/l) was achieved in the mushroom cultured in a medium containing the plant extract than the one without the extract (1.66 g/l). There was a higher exo-polysaccharide production (with higher inhibitory activity) in the mushroom cultured in an extract inclusive medium than the one without the extract (1.20 and 0.88g/l. respectively). Minimum Inhibitory Concentration of the plant extract induced exopolysaccharides was 25mg/ml. Maximum antioxidant capacity of the mushroom species grown in an extract inclusive medium was 43.5% while that without the extract was 38.2 %. DPPH scavenging activity, Metal chelating activity, total phenol content, and total flavonoid content for the mushroom species grown in a medium containing the plant extract were 59.53%, 25.84%, 82.11 ± 0.56 mg/ml and 69.14±0.14mg/ml respectively, while those of the species grown in the ordinary medium were 9.24%, 28.23%, 71.69 ± 0.88 mg/ml and 68.10±0.33mg/ml respectively. This study has therefore; shown that extract of *Annona senegalensis* improves the antimicrobial and antioxidant activities of *G. lucidum*

INTRODUCTION

Ganoderma lucidum is a medicinal mushroom that has been used for centuries in traditional Chinese medicine for its health promoting properties (Chen *et al*., 2004). Its extract has been used world-wide as ingredients in health foods, herbal medicines and dietary supplements and have been used as anti-cancer and antioxidant agents. The extract has been used for prevention and treatment of various other diseases (Sliva *et al*., 2003). Such disease conditions as hypertension, bronchitis, arthritis, neurasthenia, hepatopathy, chronic hepatitis, nephritis, gastric ulcer, tumorigenic diseases, hypercholesterolemia, immunological disorders, scleroderma, cardiovascular disease, AIDS and cancer have been managed using this mushroom species (Sliva *et al*.,2003). It is suggested that its antitumor and immune enhancing properties, along with the fact that it has no cytotoxicity effect, raise the possibility that it could be effective in preventing oxidative damage and the resulting disease condition (Liu *et al*., 2002).

There are reports in literature to suggest that this mushroom species could be used in association with other medicinal herbs such as *Annona senegalensis*, *Annona muricata*, *Sophora flavescens* etc. in the treatment of cancer, diarrhea, and cough and for wound dressing in traditional Nigerian medical practices (Abdullahi *et al*., 2003). Recent pharmacological studies have shown that *G. lucidum* extract possess antimicrobial (Wasser, 2010) and antioxidant (Lee *et al*., 2001; Shi *et al*., 2002) properties. Studies have also shown that most antibacterial components from basidiomycetous fungi were potent against Gram positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis* etc only. However, recent works show that extracts from this mushroom species were also active against Gram negative organisms like *Escherichia coli* and *Proteus vulgaris*, in vitro (Lydnal, 2004).

The major groups of compounds that have been found in the extracts of *G. lucidum* are; the polysaccharides (which are the key actives component) (Zhang *et al*., 2010), ganoderic acids (Fatmawati *et al*., 2010) and triterpenes group of compounds.

The use of some traditional herbs, like the *A. senegalensis*, can also enhance the medicinal efficiency; promote the mycelia growth and polysaccharides production of *G. lucidum*. In this study, the effect of incorporation of *A. senegalensis* extract into the growth medium of *G. lucidum* on the bioactive substances produced by the mushroom was investigated.

MATERIALS AND METHODS

Mushroom sample collection and identification

The mushroom fruit body (*Ganoderma lucidum*) was collected from its natural habitat within the premises of the Federal Institute of Industrial Research Oshodi, Lagos, Nigeria (FIIRO); it was characterized and identified according to Ofodile *et al*. (2010) method. The fresh stem bark of wild custard apple (*Annona senegalensis*) known as Abo in Yoruba was obtained from Roots and Herbs Shopping Centre, Oyingbo Ultra-Modern Market in Lagos and was identified at the Herbarium Centre, Botany Department University of Lagos.

Preparation of plant crude extract

The stem bark of *A. senegalensis* was thoroughly washed with tap water to remove debris, and rinsed with distilled water. It was oven dried at a temperature of 40 –45°C for about two weeks to prevent the active compounds from being destroyed (Mustapha, 2013). The dried stem bark was milled using an electric blender to coarse powder of about 0.5 – 1mm or 0.020 – 0.039 inches. The powder (500g) was soaked in 1000ml of absolute ethanol in a sterile conical flask for 72 hours at ambient temperature with vigorous shaking at intervals of 3-4 hours (to make ethanolic extracts). The extract was filtered, first using a sterile muslin cloth and then a Whatman No. 1 filter paper and the filtrates were evaporated to dryness under reduced pressure at 70^0 C to obtain dried extracts using Awa *et al*. (2012) method. The extract was prepared for addition into broth medium.

Isolation of pure culture of *G. lucidum*

Tissue of *G. lucidum* mycelium was cultured aseptically onto a freshly prepared plate of PDA using Ofodile *et al*. (2010) method with slight modification and was incubated at 28°C for 7days. Isolates were purified on the same medium and the mycelium maintained on PDA slant at 4°C until used for the experiment.

Growth of isolate in extract containing medium

Two batches of broth medium were compounded using bacteriological peptone (2.4g), Glucose (24g), yeast extract (2.4g), KH_2PO_4 (0.6g), K_2HPO_4 (1.2g), $MgSO_4.7H_2O$ (0.6g) at a pH of 6.5 (Sood *et al*., 2013). *A. senegalensis* ethanolic extract (ASEE) was added into one batch and adjusted to pH 6.5 using 0.1M of HCl. The second batch which does not contain the plant extract served as control. The two batches were distributed in 200 ml portions into 250 ml conical flasks each and sterilized in an autoclave at 121^{o}C for 15minutes. One millimeter agar discs were cut with a sterilized cork borer from the edge of 7 day old pure culture of *G lucidum* grown on PDA plates at 28^{o}C and inoculated into each flask. The flasks were incubated at 30^{o}C using Mahendran *et al*. (2012) method.

Determination of dry cell weight (DCW).

To determine the dry cell weight (DCW), cultured broth was centrifuged at 5000rpm × *g* and filtered using a muslin cloth. The filtrate (mycelium) was dried at a temperature of 80°C and the weight of the dry cell was determined using the method of Yan-Qun and Zhi-cong. (2011). The supernatant was used for extraction of the fungus polysaccharides.

Extraction of extracellular polysaccharides (EPSs)

The supernatant obtained after centrifugation and filtration of broth during the process of dry cell weight determination was used for extraction of the fungus polysaccharides. The extracellular polysaccharides (EPS) were precipitated from the supernatant by addition of equal volume of 95% ethanol (the mixture was agitated to prevent local high concentration of the precipitate). The mixture was left at 4^0 C for 12 hours before centrifuging and the precipitate was collected in a petri plate and dried at 60^0C using Mahendran *et al*. (2012) method.

Determination of antimicrobial properties using agar-well and disc diffusion methods

Antimicrobial activity of the extracted polysaccharides was determined using disc and agar-well diffusion methods as described by Oviasogie *et al.* (2015). Clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Candida. albicans* and *H. capsulatum* obtained from the Microbiology Laboratory of the General Hospital Ikorodu Lagos were used as test organisms.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of the extracted polysaccharide was determined by dilution method using serially diluted extracts. The extracts were diluted to get series of concentrations ranging from 12.5mg/ ml to 200 mg/ ml in sterile nutrient broth. The microorganism suspension of 0.2ml was added to the broth dilutions. The broths were incubated at 37^{o}C for 24 h and were examined for the presence of growth inhibition zone (Oviasogie *et al.*, 2015) method.

Determination of Minimum Bactericidal/Fungicidal Concentration (Mbc/Mfc)

One ml sample from tubes used in MIC determination which didn't show any visible growth after the period of incubation was streaked out on nutrient agar for 24 hours for bacteria and Sabouraud Dextrose Agar for 72 hours for *C. albicans* and *H. capsulatum*, to determine the minimum concentration of the extract required to kill the organisms. The lowest concentration of the extract that indicated a bactericidal effect after incubation was regarded as the Minimum Bactericidal Concentration (MBC), while the lowest concentration that prevented the growth of *C. albicans* and *H. capsulatum* was taken as the Minimum Fungicidal Concentration (MFC) (Atukpawu, 2014).

Antioxidant Activity

Determination of Total Antioxidant Capacity (TAC)

Total antioxidant activity of crude exopolysaccharide extracts from both *Ganoderma lucidum* grown in a medium containing plant extract and the one grown in a medium without the plant extract was determined using Mahendran *et al.* (2012) method. Fourty five militer (45ml) of sulphuric acid (0.6M), 0.9942g of Sodium sulphate (28mM) and 1.235g of ammonium molybtate (4mM) were mixed together in 250ml conical flask with

distilled water, 0.1ml of various concentrations of exopolysaccharide extracts; 10, 20, 50 and 100µl respectively was dissolved in 1ml of the reagent medium for determination of total antioxidant capacity and their absorbance were read at 695nm after 15 minutes. Ascorbic acid was used as standard.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

DPPH radical scavenging activity of crude extract was determined by a spectrophotometric method based on the reduction of methanol solution of DPPH as described by khalaf (2008). One milliliter of different concentrations; (5, 10, 15, 20 and 25 µg) of extract of mushroom grown in plant extract containing potato dextrose broth, and the one grown in PDB only was added to 1ml of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes in dark environment. The absorbance was measured at 517nm by a spectrophotometer. The absorbance of a blank solution was used as the control.

Inhibition ratio (%) of free radical, DPPH, was calculated according to the formula:

$$IDA\% = 100 - \{[(A_{sample} - A_{blank}) \times 100] / A_{control}\}$$

Where IDA: inhibition of DPPH activity

A_{blank}: The absorbance of the control reaction (containing all reagents except test compounds) and

A_{sample}: The absorbance of the test compound

The tests were carried out in triplicate. Ascorbic acid was used as positive control.

Metal chelating activity on ferrous ions [Fe^{2+}]

Metal chelating activity was determined according to the method of Oke *et al*. (2009). 0.5ml of crude exopolysaccharide extract from both *Ganoderma lucidum* grown in a medium containing plant extract and the one grown in a medium without the plant extract (PDB only) was mixed with 0.05ml of 2M $FeCl_2$ and 0.1ml of 5M ferrozine. The extracts ranging from 0.5–3.5 mg/ml was diluted to the desired total volume with the solvent.

The mixture was shaken vigorously and left standing at room temperature for 10 minutes. After the mixture had reached equilibrium, the absorbance of the solution was measured in spectrophotometer at 562 nm.

The percentage of inhibition of ferrozine Fe^{2+} complex formation was calculated using the formula given below:

Scavenging effect [%] = [$A_{Control} - A_{Sample}$ x100]/ $A_{Control}$

Where = $A_{Control:}$ is the absorbance of the ferrozine–Fe^{2+} complex and

$A_{Sample:}$ is the absorbance of the test compound.

EDTA was used as control**.**

Total phenolic content

Total phenolic content of the extracts were analyzed by using Folin Ciocalteu reagent according to the method of Oke *et al*. (2009) using Gallic acid as standard. One militer (1 ml) of the extract solutions was mixed with 0.2ml of 50% Folin Ciocalteu reagent. The mixture was allowed to react for 3 minutes, and 1ml of aqueous solution of 2% Na_2CO_3 was added. The mixture was vortex vigorously and incubated for 45 minutes at room temperature and the absorbance of each mixture was measured at 760 nm. The same procedure was also applied to the standard solutions of Gallic acid (the concentration range: 15.65- 500 μg/ml). Phenolic compounds were expressed as mg of Gallic acid equivalents/g of the extracts. The Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.003x + 0.033$, $R^2 = 0.999$, Where y is absorbance at 760 nm and x is total phenolic content in the extracts of both groups of mushroom extracts and expressed in mg/ml.

Total Flavonoid Content

The total flavonoid content was determined according to the aluminum chloride colorimetric method (Acharya *et al*., 2010). The extracts (2 ml, 0.3 mg/ml) in methanol were mixed with 0.1 ml of 10% aluminum chloride hexa-hydrate, 0.1 ml of 1 M potassium acetate and 2.8 ml of deionized water. The mixture was incubated for 40 minutes at room temperature, and the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. Total flavonoid content was expressed as milligram per g of the extracts.

Rutin was used as a standard compound and the total flavonoid was expressed as mg/ml Rutin equivalent using the standard curve equation: y = 25.5x, R^2= 0.9812, Where y is absorbance at 510 nm and total flavonoid content of both sets of mushroom extract and expressed in mg/ml.

Statistical Analysis

The experiments were carried out in triplicate. The results are given as mean ± standard deviation (SD). Student's t-test was used for comparison between the means of samples and standards. A difference was considered statistically significant when $p \leq 0.05$. Correlation analysis was carried out on the antioxidant models using SPSS 14.0 Windows Statistical package.

RESULTS

Isolation of pure culture of *G. lucidum*.

The pure culture of *G. lucidum* was successfully isolated from the mushroom species obtained from the wild (Plate 1).

Extraction of the plant extract.

The ethanolic extract of *Annona senegalensis* stem bark is shown in Plate 2 while the physicochemical-profile of the extract is shown in Table 1.

Effect of *Annona senegalensis* ethanolic extracts on the growth of *Ganodoma* species.

Inclusion of the ethanolic extract of *Annona senegalensis* in the growth medium for the cultivation of *G. lucidum* affected the growth rate of the mushroom. Five grams per liter (5g/l) of *Annona senegalensis* ethanolic extract inclusion elicited the highest growth rate (Figure 1 and Plate 3A), 7 g/l gave a moderate yield of 1.98g/l dry weight (Plate 3B), while 11g/l gave the lowest yield (0.87g/l dry weight)(Plate 3C). At thirteen grams per liter (13g/l) there was no growth (Plate 3D). The mushroom species grown on a medium without inclusion of the extract (PDB alone) showed visible growth (Plate 3E).

Plate 1: Pure culture of *G. lucidum* isolated on Potato dextrose agar.

Plate 2: The dried *Annona senegalensis* ethanolic extract (ASE

Table 1: Physicochemical-profile of Ethanolic extract of *Annona senegalensis* stems bark.

S/N	Solvent used	Color	Consistency	% of yield
1.	Ethanol	Reddish brown	Non-sticky	6.29

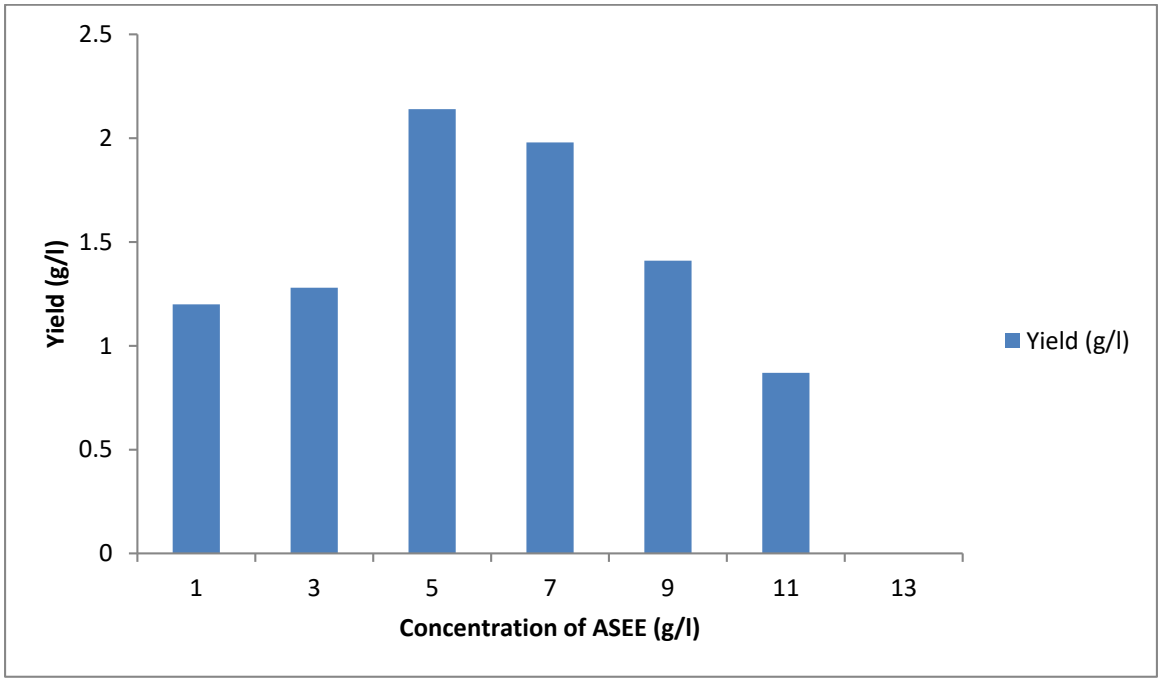
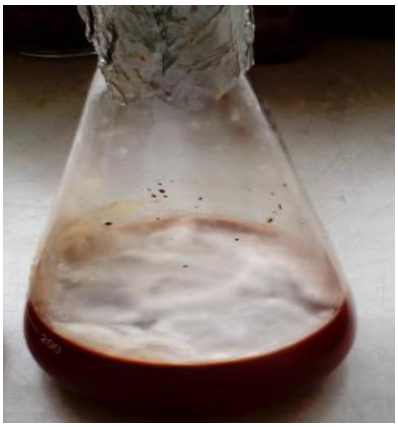


Figure 1: Effect of different concentration of *Anonna senegalensis* extract (ASEE) on the growth of *G. lucidum*



A

B

C

D

E

A- ASEE with PDB at 5g/l

B- ASSE with PDB at 7g/l

C- ASEE with PDB at 11g/l

D-ASSE with PDB at 13g/l

E- PDB only with visible growth

Exopolysaccharide (EPS) and Dried Cell Weigh (DCW) Production from *Ganoderma lucidum.*

A reddish brown crude exopolysaccharide (EPS) fraction was obtained from the mushroom species grown in a medium containing extract of *A. senegalensis* while the EPS from the one grown without the extract was pale yellow. The weight of the mycelium was weighed before and after drying. The total EPS weight before and after drying of both groups of polysaccharides were 1.89 ± 0.52 and 1.29 ± 0.43g/ml; and 1.29 ± 0.42 and 1.30 ± 0.42g/ml respectively (Table 2).

Antimicrobial activity of exopolysaccharide (EPS) extract.

The antimicrobial activity of polysaccharide extract of *G. lucidum* grown in a medium containing the plant extract and the one without the extract were tested against species of gram positive bacteria (*S. aureus*), gram negative bacteria (*E.coli*) and two fungi organisms (*C. albicans* and *H. capsulatum*), and results obtained are shown in Table 3. The highest inhibitory activity was obtained against *S. aureus* (14.00mm) while the lowest inhibitory activity was obtained against *H. capsulatum* (1.30mm).

Minimum Inhibitory Concentration (MIC) of ESPS of *G. lucidum*

Staphylococcus aureus was more susceptible to the antimicrobial activity of the EPS extract of *G. lucidum* grown in a medium containing the plant extract; with minimum inhibitory concentration of 25.0 mg/ml, than *E. coli* with MIC of 100 mg/ml. The minimum inhibitory concentration (MIC) of the extract against the tested yeast cells were the same (100 mg/ml) (Table 4). The susceptibility of the test organisms to the EPS extract of the mushroom species grown in a medium not containing the plant extract was same as observed for plant extract induced EPS (Table 5).

Table 2: Showing the DCW and EPS Production

	EPS production of *G. lucidum* grown in plant extract containing medium		EPS production of *G. lucidum* grown in medium without plant extract	
	After drying (g/l)	Before drying (g/l)	After drying (g/l)	Before drying (g/l)
1.	0.80	1.20	0.71	1.04
2.	1.19	1.28	1.03	1.15
3.	1.66	2.14	1.36	1.85
4.	1.29	1.98	1.01	1.23
5.	0.90	1.41	0.76	0.97
6.	0.23	0.87	0.21	0.58
7.	0.11	0.62	0.05	0.43
Total	0.88 ± 0.52 g/l	1.36 ± 0.51 g/l	0.73 ± 0.42g/l	1.0 4 ± 0.42g/l

Table 3: Inhibitory effect of EPS produced by *G. lucidum* on test organisms**.**

Test Organisms	EPS produced by *G. lucidum* grown in plant extract containing medium	EPS produced by *G. lucidum* grown in medium without plant extract
	Zone of inhibition (mm)	Zone of inhibition (mm)
Escherichia coli	10	9.3
Staphylococcus aureus	14	13.6
Candida albicans	12.3	10
Histoplasma capsulatum	1.7	1.3

Table 4: Minimum inhibitory concentration of EPS extract of *G. lucidum*

Test organism	Inhibitory *G.* extract	Effect *Lucidum* medium	Of grown	EPS In	Of plant	Inhibitry G. lucidu	Effect Grown	Of in	EPS PDA	
	12.5	25	50	100	200	12.5	25	50	100	200
Escherichia coli	*	*	*	+	+	*	*	*	+	+
Staphylococcus aureus	*	+	+	+	+	*	*	+	+	+
Candida albicans	*	*	*	+	+	*	*	*	+	+
Histoplasma capsulatum	*	*	*	+	+	*	*	*	+	+

*** means no inhibition; + means inhibition**

Antioxidant activity

The antioxidant activities of the mushroom species grown in a medium containing the plant extract was compared with the one grown in ordinary medium using ascorbic acid as standard. The inclusion of the plant extract in the growth medium of mushroom species significantly improved the antioxidant capacity of the mushroom. The total antioxidant capacity (TAC) of the mushroom species grown in a medium containing the plant extract was 43.5%, the one grown in ordinary medium was 38.2% and 44.3% was the TAC of the standard (Table 5).

DPPH scavenging activity at 517nm

Result obtained in this study shows that the inclusion of the plant extract in the culture medium of the mushroom species improved its DPPH scavenging activity. The mushroom species grown in a medium containing the plant had a higher scavenging activity than the one grown in ordinary medium. Highest activity was achieved at 30µg/ml for the mushroom species grown in medium containing the plant extract (59.53%), the one grown in ordinary medium had an activity of 9.24% while the control (ascorbic acid) recorded an activity level of 84.02% (Table 6). Generally, the scavenging activity increased with increase in the concentration of the extract.

Metal chelating activity at 695nm

Metal chelating activity of the mushroom species was evaluated and compared with that of a standard **(**EDTA). Result obtained indicates that at concentration of 3.5mg/ml, the scavenging activities of the mushroom species grown in ordinary medium, the one grown in plant extract containing medium and the control (EDTA) were 28.23%, 25.84% and 50.05% respectively (Table 7).

Table 5 Percentage Inhibition of Total Antioxidant Capacity (TAC)

Concentration (µg/ml)	Ascorbic acid (% Inhibition)	Mushroom grown in ordinary medium (% inhibition)	Mushroom grown in plant extract containing medium (% inhibition)
10	37.6	0.40	0.99
20	38.8	7.92	15.6
50	40.9	27.1	40.2
100	44.3	38.2	43.5

Table 6. Percentage inhibition of DPPH

Concentration (µg/ml)	Ascorbic acid (% inhibition)	Mushroom grown in ordinary medium (% inhibition)	Mushroom grown in plant extract containing medium (% inhibition)
5	2.61	0.99	0.16
10	29.66	0.65	0.98
15	80.8	0.16	10.80
20	83.01	2.65	33.75
25	83.22	2.37	41.89

30	84.02	9.24	59.53

Table 7. Percentage inhibition of Metal Chelating

Concentration (mg/ml)	EDTA(Percentage Inhibition)	Mushroom grown in ordinary medium (% inhibition)	Mushroom grown in plant extract containing medium (% inhibition)
0.5	16.06	12.25	10.5
1.0	27.64	14.96	12.89
1.5	38.8	17.56	14.78

2.0	45.09	20.05	18.25
2.5	48.34	21.09	19.65
3.0	49.56	24.89	23.47
3.5	50.05	28.23	25.84

Total Phenolic Content

Total phenolic content of the mushroom species was determined using gallic acid as the standard. The spectrophotometric absorbance of different concentrations (12.5-500µg/ml) of the standard (Gallic acid) was determined. SPSS software was used to calculate the total phenolic content of the mushroom samples from a standard curve derived from the reading of the standard. The phenolic content of the mushroom species was increased by the addition of the plant extract in the culture medium. The mean %age inhibition of free radicals by the total phenolic content for the mushroom species grown in plant extract containing medium and that grown in ordinary medium were 82.11±0.56mg and 71.69±0.88mg respectively

Total Flavonoid Content

Total flavonoid content of the mushroom species was determined using rutin as the standard. The spectrophotometric absorbance of different concentrations (2 - 4µg/ml) of the standard was determined. SPSS software was used to calculate the total flavonoid content of the mushroom samples from a standard curve derived from the reading of the standard. The flavonoid content of the mushroom species was increased by the addition of the plant extract in the culture medium. The mean %age inhibition of free radicals by the total flavonoid content for the mushroom species grown in plant extract containing medium and that grown in ordinary medium were 69.14±0.14mg and 68.10±0.33mg respectively.

Discussion

Ganoderma lucidum is a known medicinal mushroom and it is a very important mushroom in the pharmaceutical and medical fields. In this work, the morphological identification of *G. lucidum* colony was determined on potato dextrose agar plate. During the formation of this mushroom, it was observed that the mushroom grows radially without making aerial hyphae during development and it develops parallel to the

surface of the culture plate and gains an intensive cover, which at the beginning has white color pigmentation. This finding is in agreement with what Stamets (2000) reported.

The results obtained in this study show that inclusion of the ethanolic extract of *Anonna senegalensis* plant in the culture medium for growing *Ganoderma lucidum* positively affected the growth rate of the mushroom. Five gram per liter (5g/l) inclusion of this plant extract into the medium for cultivation of this mushroom elicited the highest growth rate. Further increase in the level of inclusion of this extract in the culture medium resulted in decrease in the growth rate of the mushroom. This result is in agreement with the observation of Yan-Qun Li and Zhi-Cong Zhi (2012) who studied the effect of water extract of S*ophora flavescens* on the growth of *G. lucidum.* These authors noted that the biomass growth of *Ganoderma lucidum* and its exopolysaccharide production were promoted by their plant extract. They however, observed that there was no growth as the concentration of their plant extract increased to 40g/l after 40 days of observation.

The antimicrobial activities of extract of *G. lucidum* grown with plant extract and the one grown without the extract showed that both inhibited the growth of both Gram-positive and Gram-negative bacteria as well as fungi species. The minimum inhibitory concentrations (MIC) (which is taken to be the lowest concentration of each extract that did not give any visible bacterial growth) indicate that the mushroom species grown with inclusion of plant extract gave a lower MIC than the one grown without the extract. This implies that the extract of *G. lucidum* grown with inclusion of plant extract exhibited stronger inhibitory power than the one grown without the extract. This result is in agreement with the report of Quereshi *et al*. (2010), though their MIC value was lower than what was recorded in this work. The minimum bactericidal concentrations of the extracts of both groups of mushroom (i.e., the one grown in a medium containing plant extract and the one without the extract) followed the same trend as seen with MIC result. The extract from the mushroom grown in the medium with plant extract inclusion exhibited a stronger bactericidal effect than the one without the plant extract (at 25 mg/ml and 100 mg/ml respectively). This result is supported by the report of Oviasogie *et al*. (2015), Stamets (2002) and Moradali *et al*. (2006) who reported similar result using the same mushroom species.

This study evaluated the total antioxidant capacities (TAC) of mushroom species grown in a medium containing *Anonna senegalensis* extract, that grown in ordinary medium and that of a control (ascorbic acid), and the results obtained were 49.5%, 38.2% and 44.3% inhibition of free radicals respectively. DPPH scavenging assay was conducted on the cultivated mushroom species. The DPPH radical scavenging assay is a widely accepted model to assess free radical scavenging activity. The ability of antioxidants to scavenge DPPH is attributed to their hydrogen donating activity. The scavenging effect of our mushroom extracts and that of the control increased as concentration increased. This means that at higher concentration, more free radicals were captured by DPPH resulting in decrease in absorbance in percentage inhibition value. This possibly means that extracts of the mushroom species grown in plant extract medium and the one grown in ordinary medium were both rich in antioxidant components such as proteins, amino acid, ascorbic acid which contributes to their antioxidant activities. This finding is supported by the report of Lin *et al*. (1999) who worked on methanolic extracts from other medicinal mushrooms that were extremely effective in inhibiting the lipid peroxidation.

Metal chelating capacity is important since it reduces the concentration of transition metals that may act as catalysts to generate the first few radicals and initiate the radical mediated oxidative chain reaction in biological systems. In this study, the chelating capacity for Fe^{2+} by mushroom grown in a medium containing plant extract and the one grown in ordinary medium were evaluated with EDTA as a positive control. The antioxidant capacity of mushroom grown in a medium containing plant extract was higher than the one grown in ordinary medium. This result is in agreement with the observation of Jeena *et al*. (2014) who reported that the methanol extract of some cultivated Oyster mushroom (*pleurotus* species) has greater chelating activity than the one without the extract.

In this study, total phenolic content of the mushroom grown in a medium containing plant extract and the one grown in ordinary medium were evaluated. The total phenolic content found in the mushroom grown in a medium containing plant extract and the one grown in ordinary medium were 82.67±0.56mg and 71.33±0.88mg respectively. Both samples were rich in phenol content. However, the phenol compounds found in mushroom

grown in a medium containing plant extract was higher than the one grown in ordinary medium. This finding is in agreement with the record of Yegenoglu *et al*. (2011) who reported that the total phenolic contents of ethanol and water extracts of *G. lucidum* were significantly higher than those of *F. trogii* that was grown without plant extract. The plant extract also improved the total flavonoid content of our experimental mushroom species.

The study has demonstrated that the incorporation of *Annona senegalensis* ethanolic extract on the growth medium of *G. lucidum* improves a broad spectrum of biological activities in the mushroom. However, more work still need to be done, especially with regards to isolating active compounds present and determining their mechanism of actions, biochemical and physiological effects.

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