Antioxidant, antimicrobial and phytochemical properties of alcoholic extracts of *Cantharellus cibarius* – a Nigerian mushroom

Aina, DA.[1] Jonathan, SG [2], Olawuyi, OJ.[2], Ojelabi, DO[3] and Durowoju, BM[1]

[1] Department of Biosciences & Biotechnology, Babcock University, Ilisan-Remo, Ogun State.Nigeria
[2] Department of Botany & Microbiology, University of Ibadan.oyo State Nigeria
[3] Quality Asurance Department, Pharma Deko Plc. Agbara, Ogun state, Nigeria
*Corresponding author: gbolyjoe@yahoo.com

Abstract: The alcoholic (methanolic and ethanolic) extracts of *Cantharellus cibarius* were evaluated for their antioxidant, antimicrobial and phytochemical potentials. Both extracts were found to contain phenols, terpenoids, flavonoids, alkaloids, anthraquinones and saponin. Both extracts had antioxidant activity, which was determined by DPPH radical scavenging activity assay. Methanolic extract of *C. cibarius* had an EC_{50} value of 2.68 mg/ml which presents a greater antioxidant capacity than the ethanolic extract with an EC_{50} value of 3.12 mg/ml making the methanolic extract a better scavenger of radicals. Both extracts inhibited the growth of *Escherichia coli* and *Candida albicans*, while methanolic extract possessed the ability to inhibit the growth of *Salmonella typhi* using the agar well diffusion technique. This indicated a broader spectrum of activity by the methanolic extract. Again, the ethanolic extract possessed greater antifungal activity with MIC value of 10 mg/ml compared to methanolic extract which gave 30 mg/ml. Results from this study indicate that methanol is superior to ethanol in the exudation of bioactive compounds from the fruiting body of *C. cibarius*. However, both extracts of *C. cibarius* possess bioactive metabolites and phytochemicals capable of scavenging free radicals and inhibiting the growth of microorganisms which suggests their potential for medicinal purposes.
[Aina, DA. Jonathan, SG, Olawuyi, OJ, Ojelabi, DO and Durowoju, BM. **Antioxidant, antimicrobial and phytochemical properties of alcoholic extracts of *Cantharellus cibarius* – a Nigerian mushroom.** *N Y Sci J* 2012;5(10):114-120]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 18

Keywords: *Cantharellus cibarius*, organic extracts, phytochemicals, antioxidant and antimicrobial

1.0 Introduction

Mushrooms are macrofungi with distinctive fruiting bodies and reproductive structures (Pilz *et al.*, 2003; Prasad and Wesely, 2008). Higher fungi have been identified as a major source of biologically active natural products which provide varieties of active secondary metabolites (Jonathan and Fasidi,2003;Barranco *et al.*, 2010;Olawuyi *et al,*Aina *et al*,2012). About 10,000 of the known species belong to the class basidiomyctes of which about 5,000 species are edible, while over 1,800 species are considered to have medicinal properties (Chang, 1995).

They are highly nutritive, low-calorie food with good quality proteins, vitamins and minerals (Khatun *et al.*, 2012, Jonathan *et al*,2012a). Mushrooms have continued to generate a lots of interest particularly in its consumption as food, in cure of diseases, in biodegradation and as important items of commerce in Nigeria and all over the world (Egwin *et al.*, 2011.Jonathan *et al*,2012b). Mushrooms represent a major and as yet largely untapped source of potent pharmaceutical products (Gbolagade and Fasidi,2005;Joseph *et al.*, 2009,Oluranti *et al*.,2012).

Cantharellus cibarius is an edible mushroom with high nutritive value and can be safely collected and consumed because it is easily identified (Pilz *et al.*, 2003). Commonly called golden chanterelle (Vishwakarma *et al.*, 2011), *C. cibarius* has been reported as a source of phytochemicals and antioxidants with potential medicinal values (Barros *et al.*, 2008; Egwin *et al.*, 2011). The medicinal values of plants have claimed to lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body (Phan *et al.*, 2001), is suggestive of the same phenomenon in mushrooms.

Antioxidants are chemicals with the property to neutralize or scavenge free radicals or reactive oxygen species (ROS) (Bjelakovic *et al.*, 2008). Free radicals are unstable, highly reactive, chemically incomplete substances that remove electrons from other molecules, consequently causing damage to chemicals in the body such as enzymes, making them less effective (Acworth and Bailey, 2000). Free radicals are engendered by normal metabolic activity as well as lifestyle factors such as smoking, exercise, and diet (Barros *et al.*, 2008). When present in the body, free radicals can damage tissues and delicate cell membranes. They can also damage DNA, which may lead to the initiation of certain cancers (Acworth and Bailey, 2000). The antimicrobial property of *C.*

cibarius has also been reported (Barros *et al.*, 2008). This study was set out to examine comparatively the phytochemical constituent, antioxidant and antimicrobial properties of ethanolic and methanolic extracts of *C. cibarius*.

2.Materials and Methods

2.1 Collection of mushroom

Dried fruiting bodies of *C. cibarius* bought from Oyingbo market in Lagos were identified by Dr.Gbolagade Jonathan of Botany and Microbiology Department, University of Ibadan, Nigeria.

2.2 Preparation of *C. cibarius* Extracts

The dried fruiting bodies were ground into powder before extraction. Powdered fruiting body (50g) was soaked in 500ml of absolute ethanol and absolute methanol respectively for 24 hours and filtered through Whatman no1 filter paper according to the procedure described by Barros *et al.*, (2008). The combined methanolic and ethanolic extracts were concentrated using a rotary evaporator and then evaporated to dryness at 40ºC. The methanolic extract was reconstituted in methanol for antioxidant activity assays and in DMSO for antimicrobial activity assays, at a concentration of 50 mg/mL, while ethanolic extract was reconstituted in ethanol for antioxidant activity assays and in DMSO for antimicrobial activity assays. All extracts were stored at 4°C prior to assays.

2.3 Phytochemical Screening

Chemical test was carried out on the methanolic and ethanolic extracts of *C. cibarius* to qualitatively determine the presence of some selected phytochemicals using standard procedures described by Harborne (1973) and Sofowora (1993).

2.4 Sources of Microorganisms

Bacteria isolates were collected from the Department of Biotechnology and Molecular Biology of the Nigerian Institute of Medical Research (NIMR), Yaba-Lagos, while *Candida albicans* was obtained from the standard fungi strains from the University of Fort Hare South Africa. Stored isolate of *Candida albicans* was recovered by subculturing on Saboraud Dextrose Agar and incubated at 30ºC for 24 hours, while the stored bacterial cultures were recovered by subculturing the isolates on Mueller Hinton Agar and incubated at 37ºC for 24 hours, except *Streptococcus pneumoniae* which was subcultured on Chocolate Agar and incubated at 37ºC for 24 hours. Bacterial isolates collected include *Enterococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *S. paratyphi* A, *Staphylococcus aureus*, and *Streptococcus pneumoniae.*

2.5Antimicrobial assay of *C. cibarius* extracts

Agar-well diffusion technique was employed to test for the antimicrobial activity of the methanolic and ethanolic extracts of *C. cibarius* according to the procedure of Iwalokun *et al*.(2007). Colonies of each isolate with exception of *Streptococcus pneumoniae* were emulsified in 10 ml of sterile normal saline to obtain a turbidity equivalent to 0.5 McFarland. Colonies of *Streptococcus pneumoniae* were emulsified in 10 ml of sterile normal saline to obtain a turbidity equivalent of 1.0 McFarland. Normal saline (0.1ml) containing a particular isolate was then pipette on solidified media in Petri dishes containing Chocolate Agar for *Streptococcus pneumoniae*, Mueller Hinton Agar for other bacteria isolates and Potato Dextrose Agar for *Candida albicans*. A spreader was used to spread the normal saline over the entire surface of the media to attain a confluent growth. Wells were made in the agar using a sterile cork borer and filled with 0.2 ml of 50 mg/ml and 25 mg/ml of *C. cibarius* extracts, in such a way that methanolic extract was tested against the same microorganisms as the ethanolic extract at the different concentrations. The plates were left in a dry environment for one hour to allow extracts diffuse into the agar, after which the plates containing bacteria were incubated at 37ºC for 24 hours, while plates containing *Candida albicans* were incubated at 30ºC for 24 hours. Antibiotics sensitivity discs were placed in plates containing bacteria to serve as control. A well containing 0.2 ml of Dimethylsulfuroxide (DMSO) was generally used as a positive control well in every assay. Growth inhibition was measured as diameters of zones of inhibition to the nearest 0.1mm.

2.6 Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the methanolic and ethanolic of *C. cibarius* extracts were determined by broth dilution method (Iwalokun *et al*., 2007) with modification. The extracts were constituted with DMSO into concentrations ranging from 1–25 mg/ml.

1.0 ml of each concentration was dispensed into test tubes containing 1.0 ml of nutrient or potato dextrose broth. Normal saline (0.1ml) containing bacterial or fungal isolate that had inhibited growth due to the presence of the extracts at a turbidity equivalent to 0.5 McFarland was inoculated into the test tubes. The tubes were mixed, corked with cotton wool and incubated at 37ºC for bacteria, while *C. Albicans* was incubated for 30ºC. After incubation, the tubes were then examined for microbial growth.

2.7Antioxidant assay

This was carried out according to the DPPH spectrophotometric method described by Barros *et al.* (2008). The 0.1and 0.2 mg/ml concentrations of methanolic and ethanolic extracts of *C. cibarius* (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorbance at 517 nm. The Radical Scavenging Activity was calculated as a percentage of DPPH discoloration using the equation

$$\% \text{ RSA} = [(A_{\text{DPPH}} - A_S)/A_{\text{DPPH}}] \times 100$$

Where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

The extract concentration providing 50% of RSA (EC_{50}) was calculated from the graph of RSA percentage against extract concentration.

2.8 Statistical Analysis

Data obtained were analyzed by one-way analysis of variance, while means were seperated by LSD's tests uing SPSS 15.0 version

3.0 Results

The phytochemicals present in both extracts were the same. Methanolic and ethanolic extract of *C. cibarius* were positive for the presence of flavonoids, alkaloids, anthraquinones, terpenoids, saponin and phenols, while tannins, phlobatannins, and steroids gave negative results which indicated there absence in the extracts. Table 1 shows the result of the phytochemical screening test carried out, indicating the abundance of phenols and terpenoids present in the extract compared with other phytochemiclas found to be present in the extracts

Table 1: Phytochemical analysis of methanolic and ethanolic extracts of *C. cibarius*

Phytochemical	Methanolic Extract	Ethanolic Extract
Tannins	-	-
Flavonoids	+	+
Alkaloids	+	+
Phobatannins	-	-
Anthraquinones	+	+
Terpenoids	++	++
Saponins	+	+
Phenols	++	++
Steroids	-	-

- indicates absence of phytochemical, + indicates presence of phytochemical in trace, ++ indicates abundant presence of phytochemical.

3.1Antimicrobial assay

Agar well diffusion technique which was employed to probe the antimicrobial properties of both methanolic and ethanolic extracts of *C. cibarius* revealed susceptibility of some human pathogens to both extracts indicating their antimicrobial potency. The methanolic extract inhibited the growth of *E. coli*, *S. typhi* and *C. albicans* with an MIC of 5 mg/ml, 10 mg/ml and 30 mg/ml respectively, which is represented in Table 2. The ethanolic extract inhibited the growth of *E. coli* and *C. albicans* with an MIC of 35 mg/ml and 10 mg/ml respectively, which is represented in Table 3. Both extracts were unable to inhibit the growth of other isolate with which they were tested which was observed by the absence of inhibitory zones.

Table 2: Antimicrobial activity of methanolic extract of *C. cibarius* on selected pathogens

	50mg/ml	25mg/ml	MIC
Enterococcus faecalis	NI	NI	ND
Escherichia coli	15mm	12mm	5mg/ml
Proteus mirabilis	NI	NI	ND
Salmonella typhi	18mm	14mm	5mg/ml
Salmonella paratyphi A	NI	NI	ND
Staphylococcus aureus	NI	NI	ND
Streptococcus pneumonia	NI	NI	ND
Candida albicans	15mm	NI	30mg/ml

NI indicates no zone of inhibition, ND indicates not determined.

Table 3: Antimicrobial activity of ethanolic extract of *C. cibarius* on selected pathogens

	50 mg/ml	25 mg/ml	MIC
Enterococcus faecalis	NI	NI	ND
Escherichia coli	15 mm	12 mm	5 mg/ml
Proteus mirabilis	NI	NI	ND
Salmonella typhi	18 mm	14 mm	5 mg/ml
***Salmonella paratyphi* A**	NI	NI	ND
Staphylococcus aureus	NI	NI	ND
Streptococcus pneumonia	NI	NI	ND
Candida albicans	15 mm	NI	30 mg/ml

NI indicates no zone of inhibition, ND indicates not determined.

Antioxidant assay

The DPPH radical scavenging activity assay as described by Barros *et al.*, 2008, revealed that both methanolic and ethanolic extracts of *C. cibarius* are free radical scavengers. The percentage radical scavenging activity (% RSA) at 2 different concentrations of both extracts as represented in Fig. 1 and 2 indicated that methanolic extract of *C. cibarius* had higher radical scavenging activity (antioxidant activity) at both concentrations than the ethanolic extract. A graph was drawn manually to determine the EC_{50} of the extracts which gave EC_{50} values of 2.68 mg/ml and 3.12 mg/ml for methanolic and ethanolic extracts respectively.

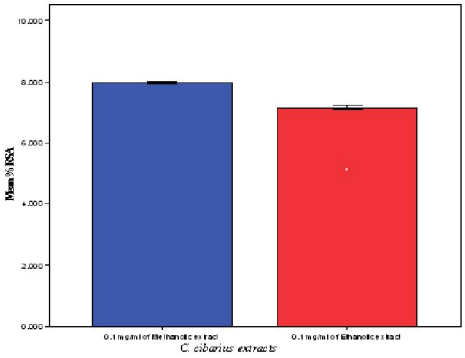

Figure 1: Percentage Radical Scavenging Activity of 0.1 mg/ml of Methanolic and Ethanolic Extracts of *C. Cibarius*.

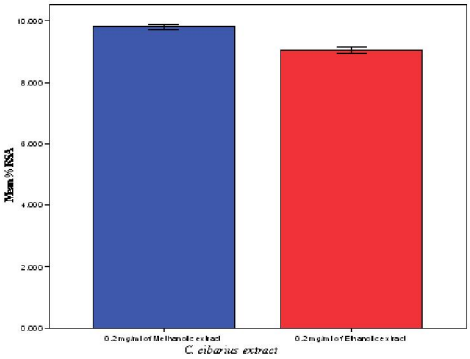

Figure 2: Percentage Radical Scavenging Activity of 0.2 mg/ml of Methanolic and Ethanolic Extracts of *C. cibarius*.

Discussion

C. cibarius, a chanterelle is popularly consumed for its nutritive value and its unique flavour (Pilz *et al.*, 2003). This research work has revealed the antimicrobial and antioxidant potency of extracts derived from *C. cibarius*, consequently suggesting its potential use in the pharmaceutical industry in making drugs with little or no side effect as it is produced from a natural source when compared to synthetic drugs. The synergistic effect of the phytochemicals and nutraceuticals present in a mushroom has been implicated in its antimicrobial and antioxidant activities (Barros *et al.*, 2008), which in the case of *C. cibarius* will be its constituent phytochemicals and nutraceuticals (i.e. pleuromutilin and chanterellins).

This study investigated the phytochemical constituent, antimicrobial activity, and antioxidant capacity of methanolic and ethanolic extracts from the fruiting body of C. cibarius. Results of phytochemical screening showed that phenols, flavonoids, saponins, alkaloids, anthraquinone, and terpenoids were present in both methanolic and ethanolic extracts. Saponins detected in both extract have been documented to strengthen the contraction of the heart muscles (Egwin *et al.*, 2011). Flavonoids and Phenols detected have been reported to provide protection against oxidative stress induced diseases and are majorly responsible for the antioxidant activity of the mushroom (Barros *et al.*, 2008; Egwin *et al.*, 2011). The presence of alkaloids also suggests the importance of the mushroom in the pharmaceutical industry as alkaloids have been reported to have a stimulating effect, act as topical anaesthetic in Ophthalmology, powerful pain reliever, antipuretic action among other uses (Egwin *et al.*, 2011). The presence of these phytochemicals indicates the importance of *C. cibarius* in the pharmaceutical industry.

The antimicrobial assay of the ethanolic extract of *C. cibarius* showed its ability to inhibit Gram positive and Gram negative bacteria as well as fungi tested *in vitro* and this suggest that *C. cibarius* has a broad-spectrum antibacterial and antifungal activity as similarly reported by Dulger *et al.*(2004), while, the methanolic extract of *C. cibarius* has been reported to inhibit the growth of only Gram positive bacteria, indicating its narrow- spectrum of antimicrobial activity (Barros *et al.*, 2008). The present study has revealed the ability of the methanolic extract of *C. cibarius* to inhibit the growth of Gram negative bacteria and *C. albicans*, which is in correlation with a previous work reported by Ramesh and Manohar (2010). Methanolic extract of *C. cibarius* was observed to inhibit the growth of *E. coli* and *S. typhi* even at a concentration of 25 mg/ml while it was effective against *C. albicans* at a concentration 50 mg/ml with an MIC of 30 mg/ml. While the ethanolic extract of *C. cibarius* was observed to inhibit the growth of *C. albicans* as low as 10 mg/ml, it inhibited the growth of *E. coli* at a concentration of 50 mg/ml with an MIC of 35 mg/ml. Antimicrobial activity of both extract is similar but a difference lies in the inability of the ethanolic extract to inhibit the growth of *S. typhi* at any concentration which is in correlation with the previous work on the ethanolic extract reported by Dulger *et al.* (2004). This suggests that methanol was able to extract slightly more bioactive compounds from the fruiting body of *C. cibarius* than ethanol with methanol having a higher dielectric constant of 33 and that of ethanol being 24.55. Similarly, Dulger *et al.* (2004) reported a decrease in spectrum of antimicrobial activity from ethanolic extract to chloroform and ethyl acetate extracts which is directly proportion to the dielectric constant value of the solvent. This indicates the ability of low polar solvents to extract bioactive compounds from *C. cibarius*, but this ability decreases with reduction in the dielectric constant of the solvent. The larger zones of inhibition observed for the methanolic extract also indicated that methanolic extract of *C. cibarius* is more effective for antimicrobial substance than the ethanolic extract. However, both extracts of *C. cibarius* were observed to be less effective than antibiotics on the antibiotics sensitivity disc. Terpenoids have been implicated as the phytochemical responsible for the antibacterial activity of *Cuminum cyminum* and *Carum carvi* (Iacobellis *et al*., 2005). Phenols and tannins have also been found to elicit antibacterial activities in many medicinal plants with mechanisms of action characterized by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhensins (Cowan, 1999). In the absence of tannins in the methanolic and ethanolic extracts of *C. cibarius*, it can thus be suggested that the abundance of terpenoids and phenols in the extracts are responsible for their broad-spectrum antimicrobial activity. Iwalokun *et al*. (2007) reported insensitivity of 6 of 8 *L. Acidophilus* and 3 of 6 *P. aeruginosa* isolates tested to *P. Ostreatus* extracts and concluded that strain variation may determine susceptibility of bacteria to antibacterial agents from mushrooms. Similarly, the observed insensitivity of *S. aureus* isolate tested against *C. cibarius* extracts which is not in correlation with results observed by Barros *et al.* (2008) and Ramesh and Manohar (2010) implies that strain variation determines susceptibility of bacteria to antibacterial agents derived from *C. cibarius*. Difference in the MICs of both extracts also suggests that methanolic extracts of *C. cibarius* is more active in its antibacterial activity compared to that of the ethanolic extract, while the ethanolic is more active in its antifungal activity compared to the methanolic extract.

The present study also observed *C. cibarius* extracts to elicit antioxidant capacity using the DPPH radical scavenging activity assay, thus expanding its nutraceutical values. The antioxidant capacity of *C. cibarius* was observed to be very low compared to 6 other species of wild Portuguese mushroom reported by Barros *et al.* (2008). The present study also observed a low antioxidant capacity for both extracts, with the huge difference in the EC_{50} values for DPPH radical scavenging activity of both extracts (2.68 mg/ml for methanolic extract and 3.12 mg/ml for ethanolic extract) and 219.31 µg/ml for ascorbic acid, a well known and very active antioxidant, as reported by Abalaka *et al.* (2011). However, methanolic extract proved to be a better scavenger of radicals than the ethanolic extract which is indicated by its lower EC_{50} value, showing the superiority of methanol in the exudation of bioactive compounds responsible for the antioxidant capacity of *C. cibarius* over ethanol. Methanolic extract of *C. cibarius* has also been reported to possess tocopherols and phenols as its active compounds responsible for its antioxidant capacity (Barros *et al.*, 2008), which conforms with the present study that observed abundance of phenols compared to flavonoids in the phytochemical screening in both extracts.

Correspondence to:
Dr Jonathan Segun Gbolagade Department of Botany & Microbiology, University of Ibadan, Ibadan.Nigeria.
Email: gbolyjoe@yahoo.com
Tel:2348164746758

References

1. Abalaka, M. E., Mann, A. and Adeyemo, S. O. 2011. Studies on *in-vitro* antioxidant and free radical scavenging potential and phytochemical screening of leaves of *Ziziphus mauritiana* L. and *Ziziphus spinachristi* L. compared with Ascorbic acid. *Journal of Medical Genetics and Genomics*, 3 (2): 28-34.
2. Aina DA, Oloke JK, Jonathan SG and Olawuyi OJ 2012.Comparative assessment of mycelial biomass and exo-polysaccharide production of wild type and mutant strains of *Schizophyllum commune* grown in submerged liquid medium *Nature and Science* 10(10):82-89
3. Acworth, I. and Bailey, B. 2000. Reactive Oxygen Species. In: *The handbook of oxidative metabolism*. Massachusetts: ESA Inc. Pp. 1.1-4.4.
4. Alade, P. I. and Irobi, O. N. 1993. Antimicrobial activities of crude extract of *Acalypha wilkesiana*. *J. Ethnopharmacol*, 39: 171-174
5. Baillie, J. K., Thompson, A. A. R., Irving, J. B., Bates, M. G. D., Sutherland, A. I., Macnea, W., Maxwell, S. R. J. and Webb, D. J. 2009. *QJM: Monthly Journal of Association of Physicians*, 102 (5): 341-8.
6. Bala, N., Aitken, E. A. B., Fechner, N., Cusack, A. and Steadman, K. J. 2011. Evaluation of antibacterial activity of Australian basidiomycetous macrofungi using a high-throughput 96-well plate assay. *Pharmaceutical Biology*. Pp. 1-9.
7. Barranco, P. G., Ocanas, L. G., Cabrera, L. V., Carmona, M. C. S., Ocanas, F. G., Gomez, X. S. R. and Rangel, R. L. 2010. Evaluation of antioxidant, immunomodulating, cytotoxic and antimicrobial properties of different strains of Basidiomycetes from Northeastern Mexico. *Journal of Medicinal Plants Research*, 4 (17): 1762-1769.
8. Barros, L., Venturini, B. A., Baptista, P., Estevinho, L. M. and Ferreira, I. C. F. R. 2008. Chemical composition and biological properties of Portuguese wild mushroom: A comprehensive study. *J. Agric. Food Chem*, 56: 3856-3862.
9. Bazzano, L. A., He, J. and Ogden, L. G. 2001. Legume consumption and risk of coronary heart disease in US men and women: NHANESI epidemiologic follow-up study. *Arch. Intern. Med.*, 161: 2573-2578.
10. Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G. and Gluud, C. 2008. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database of Systemic Reviews* (2): CD007176.
11. Campbell, N. A. and Reece, J. B. 2005. *Biology*. 7th ed. Pearson Education, Inc., Publishing as Benjamin Cummings, San Francisco. Pp. 618.
12. Chang, S. T. 1995. *Ganoderma* The leader in production and technology of mushroom nutriceuticals. In Proc 6th Int symp Recent Adv. *Ganoderma lucidum* Res (Ed. B. K. Kim, I. H. Kim and Y. S. Kim) pp 43-52. The pharmaceutical Society of Korea, Seoul, Korea.
13. Chang, S. T. and Buswell,J. A. 1996. Mushroom nutriceuticals. *World J. Microbiol. Biotech.*, 12: 473-476.
14. Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clinical Microbiol. Rev.*, 12 (4): 564-582.
15. Degreef, J., Malaisse, F., Rammeloo, J., and Baudart, E. 1997. Edible mushrooms of the Zambezian woodland area: A nutritional and ecological approach. *Biotechnol. Agron. Soc. Environ.*, 1 (3): 221-231.
16. Dulger, B., Gonuz, A. and Gucin, F. 2004. Antimicrobial activity of the macrofungus *Cantharellus cibarius*. *Pakistan Journal of Biological Sciences*, 7 (9): 1535-1539.
17. Egwin, E. C., Elem, R. C. and Egwuche, R. U. 2011. Proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigerian mushroom. *American Journal of Food and Nutrition*, 1 (2): 89-94.
18. Halliwell, B. 1996. Antioxidants in human health and disease. *Annu. Rev. Nutr.*, 16: 33-50.
19. Harborne, J. B. 1973. Phytochemical methods. Chapman and Hall ltd, London. Pp. 49-188.
20. Gbolagade JS and Fasidi I.O 2005.Antimicrobial activities of some selected Nigerian mushrooms .*African Journal of Biomedical Sciences*.8 (2):83-87
21. Iacobellis, N. S., Lo Cantore, P., Capasso, F. and Senatore, F. 2005. Antibacterial activity of *Cuminum cyminum L.* and *Carum carvi L.* essential oils. *J. Agric. Food Chem.*, 53: 57-61.
22. Iwalokun, B. A., Usen, U. A., Otunba, A. A. and Olukoya, D. K. 2007. Comparative phtyochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus Ostreatus*. *African Journal of Biotechnology*, 6 (15): 1732-1739.
23. Jonathan, SG. and Fasidi,I.O (2003). Antimicrobial activities of *Lycoperdon pusilum* (Bat. Ex.) and *Lycoperdon giganteum (Pers.), Nigerian edible macro fungi. African Journal Biomedical Research*.6(2):.88 – 90
24. Jonathan,S.G,Akinfemi,A and Adenipekun,C.O(2010).Biodegradation and invitro digestibility of maize husks treated with edible fungi (*Pleurotus tuber-regium* and *Lentinus subnudus)* from Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry(EJEAFche)* 9(4):742-750
25. Jonathan S.G Okon, C.B.Oyelakin A.O and Oluranti OO 2012a. Nutritional values of oyster mushroom (*Pleurotus ostreatus*) (Jacq. Fr.) Kumm cultivated on different agricultural wastes.*Nature and Science;* 10(9):186-191

26. Jonathan SG Okorie AN and Babayemi OJ Oyelakin AO Akinfemi A 2012b .Biodegradation of agricultural wastes (rice straw and sorghum stalk) into substrates of utilizable products using white rot fungus(*Pleurotus florida). Nature and Science*10(9):131-137.
27. Joseph, S., Sabulal, B., George, V., Smina, T. P., and Janardhanan, K. K. 2009. Antioxidant and anti-inflammatory activities of the chloroform extract of *Ganoderma lucidum* found in south India. *Sci. Pharm*., 77: 111-121.
28. Khatun, S., Islam, A., Cakilcioglu, U. and Chatterjee, N. C. 2012. Research on mushroom as a potential source of Nutraceuticals: A review on Indian perspective. *American Journal of Experimental Agriculture*, 2 (1): 47-73.
29. Klaus, A. and Miomir, N. 2007. Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. *Proc. Nat. Sci*., 133: 219-226.
30. Krishnaiah, D., Devi, T., Bono, A. and Sarbatly, R. 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medicinal Plants Research*, 3 (2): 67-72.
31. Krishnaiah, D., Sarbatly, R. and Bono, A. 2007. Phytochemical antioxidants for health and medicine – A move towards nature. *Biotechnol. Mol. Biol. Rev*., 1(4): 97-104.
32. Lenaz, G. 2001. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB Life,* 52 (3-5): 159-64.
33. Middleton, E. Jr., Kandaswami, C. and Theoharides, T. C. 2000. The effects of plant flavonoids on mammalian cells, implication for inflammation, heart disease and cancer. *Pharmacology,* 52: 673-751.
34. Mizuno, T. 1993. Food function and medicinal effect of mushroom fungi. *Foods Food Ingred J*., Pp 55-70.
35. Okeke, E. C., Eneobong, H. N., Uzuegbunam, A. O., Ozioko, A. O. and Kuhnlein, H. 2008. Igbo traditional food system: documentation, uses and research needs. *Pakistan Journal of Nutrition*, 7 (2): 365-376.
36. Olawuyi O.J;Jonathan SG,Aina DA and Baysah GI 2010.Studies on antibacterial activities of *Fomes lignosus*-A Nigerian mushroom. *Nigerian Journal of Microbiology* 24(1):2153-2159
37. Oluranti O.O,Olawuyi o.J and Jonathan S.G2012. Therapeutic properties of some Nigerian higher fungi. *Nature and Science* 10(10):135-143
38. Persson, O. and Mossberg, B. 1997. *The chanterelle book*. Berkeley, CA: Ten Speed Press. Pp. 9
39. Phan, T. T., See, P. O., Lee, S. T. and Chan, S. Y. 2001. Antioxidant effects of the extracts from the leaves of *chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine-xanthine oxidase induced damage. *Burns,* 27 (4): 319-27.
40. Pilz, D., Norvell, L., Danell, E. and Molina, R. 2003. Ecology and management of commercially harvested Chanterelle mushrooms. *Gen. Tech. Rep. PNW-GTR-576. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station*. Pp. 1-16.
41. Prasad, Y. and Wesely, W. E. G. 2008. Antibacterial activity of the bio-multidrug (*Ganoderma lucidum*) on Multidrug resistant Staphylococcus aureus (MRSA). *Advanced Biotech*., 10: 9-16.
42. Quereshi, S., Pandey, A. K. and Sandhu, S. S. 2010. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. *People's J. Scientific Res*., 3 (1): 9-14.
43. Ramesh, C. and Pattar, M. G. 2010. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of western ghants of Karnataka, India. *Pharmacog. Res*., 2 (2), 107-112.
44. Sies, H. 1997. "Oxidative stress: oxidants and antioxidants". *Exp Physiology,* 82 (2): 291-295.
45. Sofowora, A. 1993. *Medicinal plants and traditional medicine in Africa*. 2nd ed. Spectrum books, Ltd., Ibadan, Nigeria. Pp. 150.
46. Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I. and Bahorun, T. 2005. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat. Res*., 579: 200-213.
47. Valentao, P., Andrade, P. B., Rangel, J., Ribeiro, B., Silva, B. M., Baptista, P. and Seabra, R. M. 2005. Effect of the conservation procedure on the contents of phenolic compounds and organic acids in Chanterelle (*Cantharellus cibarius*) mushroom. *J. Agric. Food Chem*., 53: 4925-4931.
48. Vishwakarma, M. P., Bhatt, R. P., and Gairola, S. 2011. Some medicinal mushrooms of Garhwal Himalaya, Uttarakhand, India. *Int. J. Med. Arom. Plants*, 1(1): 33-40.

8/20/2012