Journal of Applied Biology & Biotechnology Vol. 7(04), pp. 32-38, July-August, 2019
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2019.70406

Pleurotus pulmonarius (Fr.) Quel. (Pleurotaceae): *In vitro* antioxidant evaluation and the isolation of a steroidal isoprenoid

Blessing Onyinye Okonkwo, Ozadheoghene Eriarie Afieroho*, Emeka Daniel Ahanonu, Lambert Okwubie, Kio Anthony Abo
Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Nigeria

ARTICLE INFO

Article history:
Received on: November 21, 2018
Accepted on: February 11, 2019
Available online: July 04, 2019

Key words:
Pleurotus pulmonarius,
antioxidants, mushrooms,
steroidal isoprenoid.

ABSTRACT

This study is reporting the *in vitro* antioxidant activity of the fruiting body of the edible mushroom *Pleurotus pulmonarius* (Fr.) Quel**.** The fruiting body of the edible mushroom was defatted with n-hexane and further extracted by successive maceration in dichloromethane (DCM) and 80% aqueous ethanol (AQE) to obtain the DCM and AQE extracts, respectively. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and iron chelating assays were used for antioxidant evaluation *in vitro* with ascorbic acid as the reference standard for comparison. Isolation and structural elucidation of the steroid were done using chromatographic and spectroscopic techniques, respectively. The trend for the DPPH radical scavenging activity: ascorbic acid [inhibition concentration $(IC)_{50}$ = 0.012 mg/ml] > AQE (IC_{50} = 0.525 mg/ml) > DCM (IC_{50} = 1.820 mg/ml) and for the iron chelating activity: ascorbic acid (IC_{50} = 0.214 mg/ml) > AQE (IC_{50} = 1.318 mg/ml) > DCM (IC_{50} = 7.586 mg/ml) were observed. From the DCM was isolated a pure compound **1** elucidated to be the known steroidal isoprenoid: ergosta-5, 7, 22-trien-3**β**-ol. This study aside reporting the isolation of the known compound **1** from this species of *Pleurotus*, has also shown that compound **1** has a significant iron chelating activity at 0.001 mg/ml of 77.06% compared to 4.56% at same concentration for DPPH scavenging activity.

1. INTRODUCTION

The importance and awareness of antioxidants are increasing because they are useful in treating and preventing diseases that arise due to oxidative stress. Antioxidant-rich foods have been found to promote longevity and good health [1]. Specific roles of antioxidants in preventing cancer and as anti-aging agents have been reported [2]. For years, mushrooms have been a source of food nutrients, as well as important bioactive compounds useful in medicine. Traditional reports on the use of extracts from *Pleurotus* species in the treatment of some illnesses are documented. *Pleurotus pulmonarius* is one of the mushrooms that are used traditionally as an anti-aging agent, in treating *Diabetes mellitus*, skin, and heart diseases among others. Several animals and *in vitro* studies have validated that most of these activities are due to its antioxidant properties [3]. Although several reports on the antioxidant activity of mushrooms cultivated mostly in Asian countries have been reported [3–5], similar report for species in Nigeria is scarce. As a follow-up to previous reports on the bio-prospection for bioactive metabolites from the mycoflora in Nigeria that could serve as leads in drugs and agrochemicals discovery and development [6–11], we are reporting in this present study the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and iron chelating activities of *P. pulmonarius* fruiting bodies extract, as well as the isolation of the antioxidant bioactive compound(s) which led to isolation of a steroidal isoprenoid.

2. MATERIALS AND METHODS

2.1. Sample Collection and Processing

Fresh fruiting bodies of *P. pulmonarius* (12 kg) were purchased from Dilomats Farm Ltd, Rivers State University of Science and Technology, Port Harcourt, Nigeria, and authenticated by Dr. C. Ekeke, the taxonomist at the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. A herbarium specimen with a voucher number UPH/V/1287; UPH/P/130 was deposited in the same herbarium. The sample

*Corresponding Author
Ozadheoghene Eriarie Afieroho, Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Nigeria.
E-mail: ozadheoghene.afieroho@uniport.edu.ng

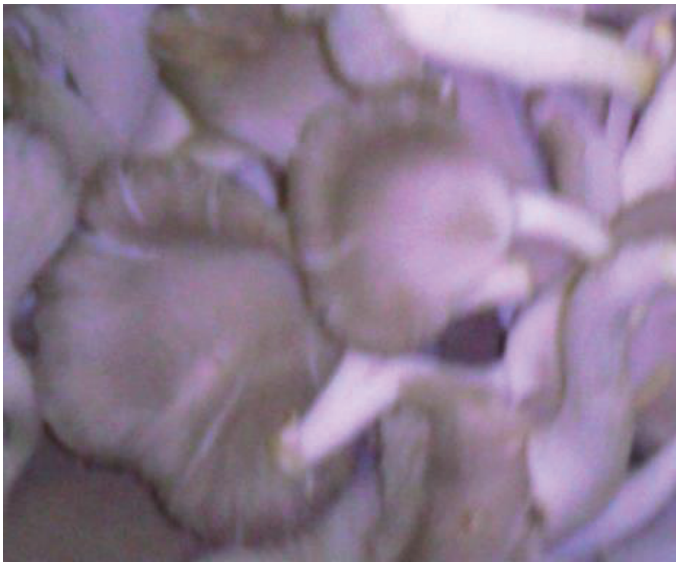

Figure 1: Fresh fruiting bodies of *P. pulmonarius.*

was cut into small portions and air-dried under a current of dry air at room temperature for 3–4 days. The dried sample was homogenized and stored in screw tight jars until use.

2.2. Sample Extraction

A 600 g of the dried powder was defatted by maceration [12] in n-hexane (6 l) at room temperature in a macerating glass bottle with intermittent agitation for 72 hours. During this period, fresh replacement of the solvent was done after every 24 hours. The defatted marc was air dried to remove residual n-hexane and further extracted by cold maceration in dichloromethane (DCM) and 80% aqueous ethanol (AQE) in succession. For each of the solvents, the maceration [12] was done for 72 hours with the fresh replacement of the solvent after every 24 hours. The dried DCM and 80% AQE extracts were obtained after using a rotary evaporator to concentrate their respective filtrate. The two extracts DCM and AQE were kept in the desiccator until further use.

2.3. Phytochemical Screening

Phytochemical screening was done using standard phytochemical screening reagents as reported previously [12,13].

2.4. Antioxidant Assays

2.4.1. DPPH radical scavenging assay

A quantitative DPPH antioxidant assay was performed on the two extracts (DCM and AQE) following the standard method [14] with some modifications. Preliminary DPPH antioxidant assay was done on the extracts (AQE, DCM, and the fatty n-hexane extract) at 0.5 mg/ml in triplicate to ascertain their degree of activity. Thereafter, six different concentrations within the range of 0.03125–1.00000 mg/ml were obtained by two-fold serial dilution, and the stock solution (10 mg/ml) were prepared for the active extracts (AQE and DCM) and used for the quantitative DPPH antioxidant assay to determine their respective inhibition concentration (IC_{50}) Briefly, with 2 ml of each of the test concentration in a test tube wrap with a foil to prevent exposure to light, 2 ml of 0.02% DPPH solution in methanol was separately added and the mixtures were shaken and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm against a blank (methanol). A solution devoid of the test extracts but containing 2 ml of the DPPH solution and 2 ml of methanol was used as a negative control while ascorbic acid was used as a reference antioxidant agent for comparison. Percentage inhibition of DPPH free radicals was calculated following the formula below:

$$\textbf{Inhibition of DPPH radical (\%)} = 100 \times \frac{\left[A_{(\text{Negative control})} - A_{(\text{sample})} \right]}{A_{(\text{Negative control})}}$$

Where: $A_{(\text{Negative control})}$ = Absorbance of the negative control solution (containing all reagents except the test extract)

$A_{(\text{Sample})}$ = Absorbance of the test extract

The IC_{50} value is the effective concentration of extract that scavenged 50% DPPH radicals and it was obtained by extrapolation from the regression curve.

2.4.2. Iron chelating assay

The extract ability to chelate the ferrous ions (Fe^{2+}) in ferrous chloride was estimated by a standard method [15]. Briefly, a 2 ml of 200 µM ferrous chloride was added separately to 2 ml of varying concentrations of the extracts (1.0, 2.0, 4.0, 6.0, and 8.0 mg/ml). The reaction was started by the addition of 0.05% o-Phenanthroline in methanol. The mixture was shaken strongly and left to stand at room temperature for 10 minutes before measuring the absorbance at 510 nm. The percentage inhibition of o-Phenanthroline–Fe^{2+} complex formation was calculated using the formula.

$$\textbf{Iron chelating potential (\%)} = 100 \times \frac{\left[A_{(\text{Negative control})} - A_{(\text{sample})} \right]}{A_{(\text{Negative control})}}$$

Where: $A_{(\text{Negative control})}$ = Absorbance of the negative control solution (containing all reagents except the test extract)

$A_{(\text{Sample})}$ = Absorbance of the test extract

The IC_{50} value is the effective concentration of the extract resulted in 50% iron chelating action and it was obtained by extrapolation from the regression curve.

2.5. Isolation and Purification of Compound 1 From the Dichloromethane Extract

The DCM extract (3.95 g) was pre-adsorbed by mixing in silica gel (4 g) and loaded on a column (internal diameter 4 cm), dry packed with silica gel (200–400 mesh, KCM light, India) to a height of 36 cm. The mobile phase gradient (500 ml of each) used comprised of n-hexane (5:0 v/v); n-hexane: DCM (4:1, 3:2, 2:3, 1:4, 0:5 v/v); DCM: ethanol (4:1, 3:2 v/v). The eluted fractions were collected at 100 ml intervals and pooled based on observed R_f of resolved spots and color reaction with chromogenic spray reagent from TLC. On the basis of these, the pooled fractions eluted with hexane/DCM 1:4 to DCM/ethanol 4:1 showed a radical scavenging activity after

Table 1: ^{1}H and ^{13}C NMR ($CDCl_3$) spectra data of compound.

Position	δ_C (ppm)	Published [18] δ_C ppm	δ_H ppm	Published* δ_H ppm	H-H COSY	HMBC
C-1	38.38	38.4	1.38; 1.80	1.28/1.80	H_2	
C-2	31.99	32.0	1.52; 1.91	1.52/1.86	H_1, H_3	
C-3	70.47	70.4	3.50	3.55		
C-4	40.80	40.8	2.32	2.25/2.49		C_5
C-5	141.37	139.8	-	-		
C-6	119.59	119.6	5.60	5.50	H_7	C_8, C_4, C_{10}
C-7	116.29	116.3	5.40	5.27		C_9, C_{14}
C-8	139.79	141.3	-	-		
C-9	46.25	46.2	1.99	1.91		
C-10	37.04	37.0	-	-		
C-11	21.12	21.1	1.70	1.64		
C-12	39.09	39.1	2.05	-		
C-13	40.41	42.8	-	-		
C-14	54.56	54.6	1.92	1.90		
C-15	23.00	23.0	1.40; 1.70	1.68		
C-16	28.30	28.3	-	1.28/1.72		
C-17	55.73	55.7	1.30	1.25		
C-18	12.06	12.0	0.65	0.66		
C-19	16.29	17.6	0.93	0.91		
C-20	40.44	40.4		2.01		
C-21	21.11	21.1	1.65	1.02		
C-22	135.58	135.6	5.20	5.07	H_{23}	
C-23	131.98	132.1	5.25	5.10	H_{20}	
C-24	42.83	42.8	2.48	1.80		
C-25	33.09	33.1		1.38		
C-26	19.96	19.8	0.86	0.89		
C-27	19.66	19.6	0.84	0.79		
C-28	17.61	17.8	0.97	0.79		

spraying with DPPH reagent and from it was isolated compound **1** after re-crystallization from acetone. Its purity was confirmed by melting point determination and from its single spot on TLC with several mobile phases. It was also evaluated for free radical and iron chelating activity at 1 mg/ml following the method outlined above.

2.6. Characterization of Compound 1

The structure of compound **1** was elucidated using spectroscopic techniques: Infrared, 1D, 2D-NMR, and mass spectrometry.

Appearance: white needle-like crystal

Molecular Formula: $C_{28}H_{44}O$

Melting point: 146°C–150°C

Phytochemistry: positive to Lieberman Burchard test

Molecular mass (observed): 396 g/mol.

FTIR [ν_{max} cm^{-1}, Nujol]: 3412 [OH str], 3060 [= CH str], 2951; 2868 [aliphatic C-H str], 1655; 1600 [C = C str, non-conjugated], 1053 [C-O str], 727 [C=C def].

^{1}H-NMR spectrum [chemical shift, δ_H, (ppm): (500 MHz, $CDCl_3$)]: See Table 1.

^{13}C-NMR spectrum [chemical shift, δ_C, (ppm): (125 MHz, $CDCl_3$)]: See Table 1

Major EI-MS m/z fragmentation peaks (relative abundance): 396 (62.66) [M^+]; 378 [M^+ - H_2O]; 363 (42.96) [M^+ - (18 + 15)]; 271 (20.45) [M^+- aliphatic chain]; 253 (30.95) [M^+ - H_2O -aliphatic chain]; 285[M^+ - H_2O - 15-ring A]; 69 (100).

3. RESULTS AND DISCUSSION

Carbohydrates, alkaloids, amino acids, glycosides, and triterpenoids/steroids were present as phytoconstituents in the dried fruiting bodies of *P. pulmonarius,* while anthraquinones and phenolics compounds like flavonoids and tannins were absent. This is in agreement with the earlier report [16] demonstrating the absence of important enzymes needed for the biosynthesis of flavonoids and related phenolics compounds in mushrooms. This is, however, contrasting report of the presence of phenolics compounds [4,5] for species outside Nigeria. The result of the *in vitro* antioxidant assays (DPPH free radical scavenging) and iron

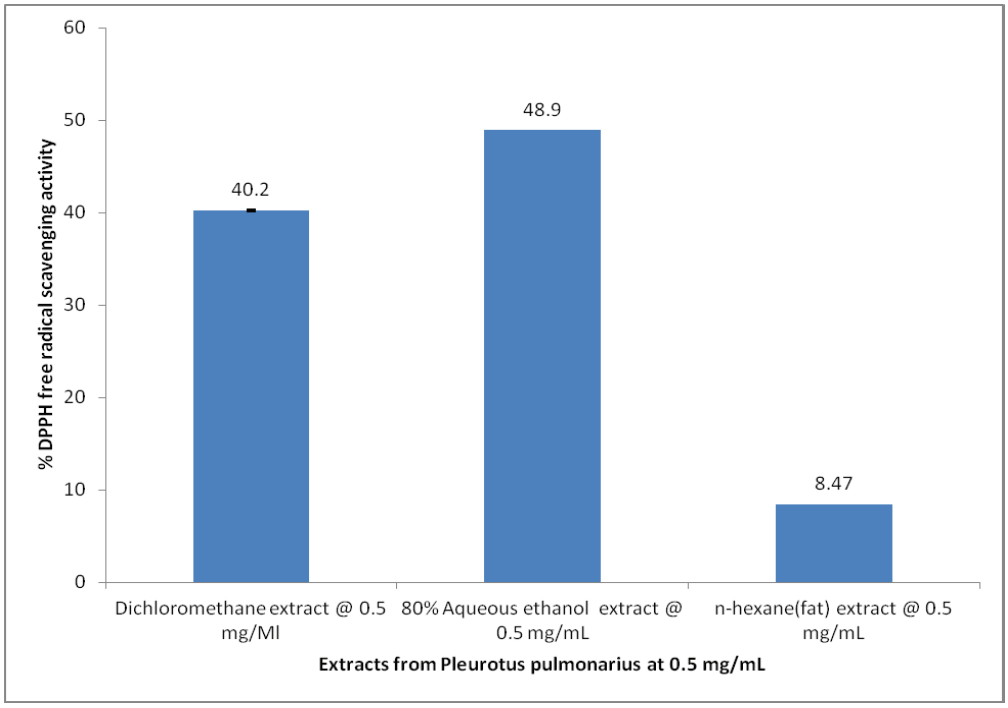

Figure 2: Preliminary *in vitro* antioxidant (DPPH) assay of the extracts from *P. pulmonarius* fruiting bodies at 0.5 mg/ml.

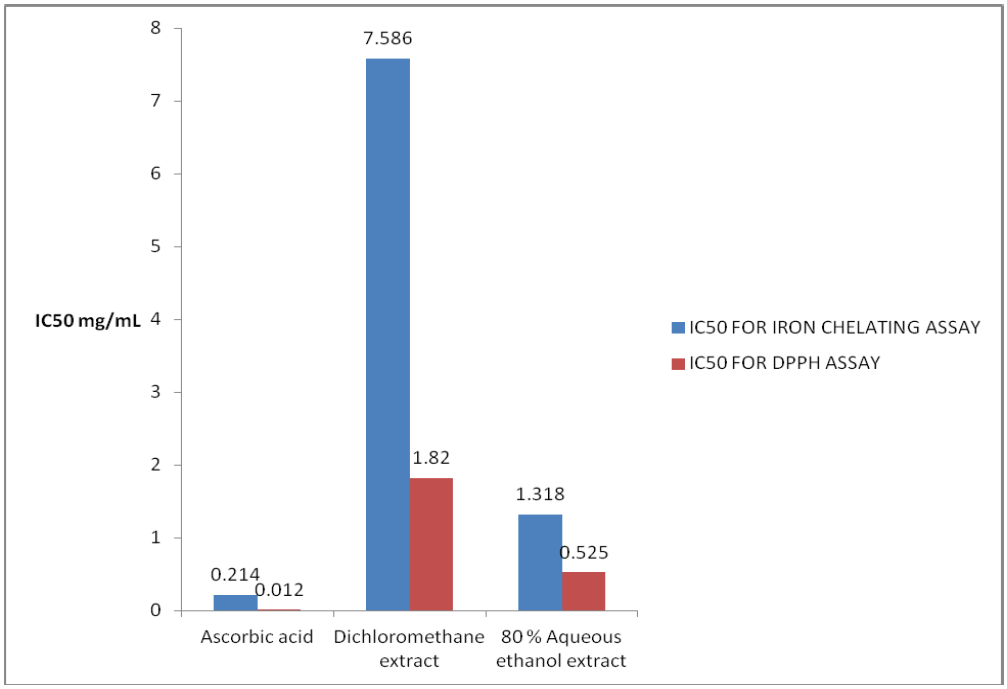

Figure 3: *In vitro a*ntioxidant profile of the lipophilic (Dichloromethane) and hydrophilic (80% AQE) extracts of *P. pulmonarius*.

chelating assays for the extracts is shown in Figures 2 and 3. The preliminary quantitative DPPH spectrophotometric assay of the extracts at 0.5 mg/ml (see Fig. 2) showed the trend in percentage inhibition of DPPH activity to be AQE (48.88%) > DCM (40.20%) > n-hexane (8.47%), which were significantly different ($p < 0.05$). After evaluating the two most active AQE and DCM for their median IC_{50}, which is the concentration that will bring about a 50% reduction in DPPH activity, the trend for the DPPH radical scavenging activity: ascorbic acid (IC_{50} = 0.012 mg/ml) > AQE (IC_{50} = 0.525 mg/ml) > DCM (IC_{50} = 1.820 mg/ml) was

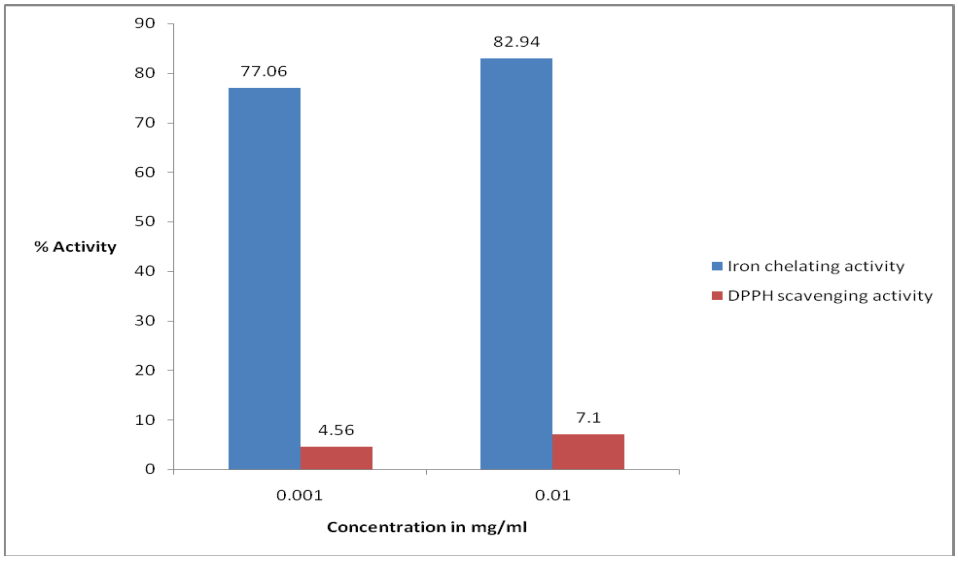

Figure 4: Dose-dependent DPPH scavenging activity and iron chelating activity of compound 1.

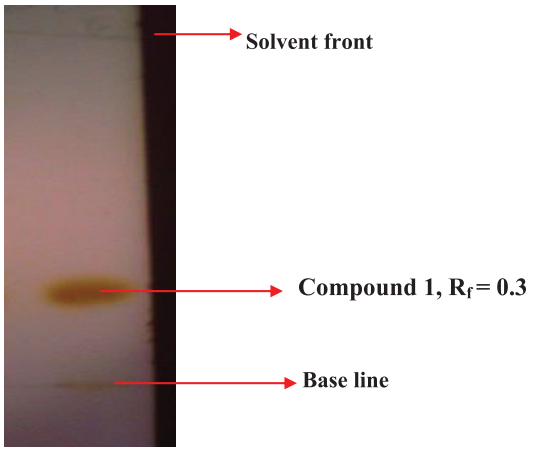

Figure 5: Thin layer chromatogram of compound 1 visualized with iodine fume. Adsorbent: pre-coated silica gel $60HF_{254}$; Mobile phase: n-hexane:dichloromethane (1:1v/v).

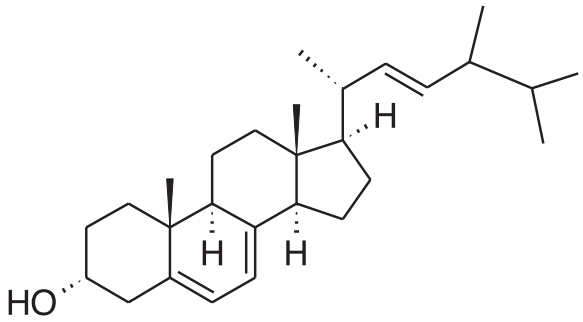

Figure 6: Structure of compound 1.

observed (see Fig. 3). Using the *in vitro* iron chelating assay as a second antioxidant assay model, a similar trend was also observed for the iron chelating activity (see Fig. 3): ascorbic acid (IC_{50} = 0.214 mg/ml) > AQE (IC_{50} = 1.318 mg/ml) > DCM (IC_{50} = 7.586 mg/ml). When compared to that for the DPPH radical scavenging activity, it showed that the antioxidant constituents in the two active AQE and DCM extracts are more of a radical scavenger than iron chelator. The result of the percentage inhibition of compound **1** using DPPH scavenging model and iron chelating activity (Fig. 4) showed that the isolated compound has a better iron chelating activity than DPPH scavenging activity. At 0.001 mg/ml, compound **1** had an iron chelating percentage inhibition of 77.06% compared to 4.56% at the same concentration for DPPH scavenging activity. Thus, it is a better iron chelator than a radical scavenger. The main mechanism of the ferrous ion chelating activity assay is the ability of *o*-Phenanthroline to deactivate and/or chelate Fe^{2+} that can promote Fenton reaction and hydroperoxide decomposition. In the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Iron is a life saver as a chemical component of heme in hemoglobin is capable of carrying oxygen throughout the body. This capability makes it physiologically essential as a useful component of cytochromes and oxygen-binding molecules. On the other hand, in a free iron (Fe^{2+}) phase, it is biochemically dangerous because it catalyzes the conversion of hydrogen peroxide to free-radical ions (especially OH), which then attack cellular membranes, protein, and DNA. Iron-related complications are reduced by chelating therapy and by doing so reduce morbidity and mortality in clinical cases of iron overload [17].

The thin layer chromatogram (Fig. 5), and the NMR data (Table 1) for compound **1** isolated from DCM extract are also reported. The FTIR spectrum gave a medium to sharp vibrational frequency in the region 3,412 cm^{-1} that indicated the presence of a hydroxyl group, typical for alcohol. This was confirmed by the proton chemical shift at 3.5 ppm in the ^{1}H-NMR spectrum. Other functional groups discernible from the FTIR spectrum are C-H stretching (2,951/2,868 cm^{-1}) of CH_2/CH_3 groups, C-O stretching (1,053 cm^{-1}) of the alcohol, and C = C stretching (1,655 cm^{-1})

of olefinic functional groups. Further confirmatory evidence for the olefinic functional group was seen from the NMR spectrum (Table 1). The chemical shifts at δ_H: 5.6 and 5.4 ppm resonated for the olefinic protons at position C-6 and C-7, respectively, while δ_H: 5.2 and 5.25 ppm resonated for that at position C-22 and C-23 of the aliphatic side chain, respectively. The proton signals for the six methyl (CH_3) at H-11, H-18, H-19, H-26, H-27, and H-28 positions resonated upfield at δ_H: 1.65, 1.70; 0.65; 0.97; 0.86; 0.84; and 0.93 ppm, respectively**.** The overlapping methine (CH) and methylene (CH_2) protons resonated at the chemical shift between δ_H: 2.5 and 1.99 ppm. The various chemical shifts in the ^{1}H–NMR were corroborated by the ^{13}C–NMR (Table 1). For instance, the signal at δ_C 70.47 ppm indicated the carbon atom at position C-3 bearing the carbinol protons in the ^{1}H–NMR, while the six deshielded carbon signals C-5 (δ_C: 141.37 ppm), C-6 (δ_C: 119.59 ppm), C-7 (δ_C: 116.29 ppm), C-8 (δ_C: 139.79 ppm), C-22 (δ_C: 131.98 ppm), and C-23 (δ_C: 135.58 ppm) are further credence to the presence of olefinic bond typical of unsaturated sterols. The DEPT-135 spectrum revealed that a total of four peaks are quaternary carbon: two olefinic carbons C-5 (δ_C: 139 ppm) and C-8 (δ_C: 141.37 ppm) and two saturated C-10 (δ_C: 37.04) ppm and C1-3 (δ_C: 40.41 ppm), while the remaining olefinic carbon: C-22 **(**δ_C: 131.98 ppm) and C-23 (δ_C: 135.58 ppm**)** are methine (CH) in nature. In all, the spectrum showed about seven methine, including the carbinol (6 × CH and 1 × CHOH), six methylene ($6CH_2$) and seven methyl (7 × CH_3), and four quaternary (4 × C). The unambiguous assignment of these positions was further done using two dimensional NMR experiment (HSQC, H-H COSY, and HMBC). In view of all the spectra and chemical information, compound **1** was proposed to be ergosta-5, 7, 22-trien-3**β**-ol. The proton and ^{13}C NMR data, when compared with that reported for ergosterol in the literature [18] were found to be in agreement. Further mass spectroscopy analysis gave a molecular ion peak at m/z 396 calculating for $C_{28}H_{44}O$ with other diagnostic fragmentation peaks at:[*m/z* (rel. int)]: 396 (62.66) [M^+], 378 [M^+ - H_2O] due to the loss of water, 363 (42.96) [M^+ - (18 + 15)] due to loss of both of water and an angular methyl group, 271 (20.45) [M^+ - aliphatic chain], 253 (30.95) [M^+ - H_2O - aliphatic chain], and 285 [M^+ - H_2O -15-ring A] rationalized. These spectra (NMR, MS, and IR) are also in agreement with those obtained for the known steroid ergosterol isolated and reported for its closely related species *Pleurotus otreatus* [6].

Ergosterol with IC_{50} (DPPH) of 0.46 mg/ml [19] and ergosterol rich extract [20] have been documented to possess antioxidant activity. Ergosterol is a major contributor to the therapeutic effect of saireito, a standardized herbal product used in Japan for the treatment of ulcerative colitis, an inflammatory disease [21]. Its usefulness in cancer prevention has also been reported [22].

4. CONCLUSION

This study aside reporting the isolation of this compound **1** from this species of *Pleurotus* has also shown that it possesses a significant iron chelating activity.

ACKNOWLEDGMENT

Ozadheoghene Eriarie Afieroho gratefully acknowledged financial support from the Royal Society of Chemistry for a training workshop traveling bursary to the Kwame Nkrumah University (KNUST), Kumasi in 2015 that brought a collaborative research initiative between his research group and the Central Instrument facility at the KNUST where the spectroscopy components of this project was done.

REFERENCES

1. Anisimov VN. Effects of exogenous melatonin—a review. Toxicol Pathol 2003;31:589–603.
2. Owen RW, Giacosa A, Hull W, Hauber R, Spiegelhalder R, Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from Olive oil. Eur J Cancer 2000;36(10):1235–47.
3. Jose N, Ajith TA, Janadharnan KK. Antioxidant, anti-inflammatory, and antitumor activities of culinary-medicinal mushroom *Pleurotus pulmonarius* (Fr.) Quel. (Agaricomycetideae). Int J Med Mushrooms 2002;4:7.
4. WW, Li B, Lai ET, Chen L, Huang JJ, Cheung AL, et al. Water extract from *Pleurotus pulmonarius* with antioxidant activity exerts *in vivo* chemoprophylaxis and chemosensitization for liver cancer. Nutr Cancer 2014;66(6):989–98.
5. Nguyen TK, Im KH, Choi J, Shin PG, Lee TS. Evaluation of antioxidant, anti-cholinesterase, and anti-inflammatory effects of culinary mushroom *Pleurotus pulmonarius*, Mycobiology 2016;44(4):291–301.
6. Afieroho OE, Siwe Noundou X, Onyia CP, Festus OH, Chukwu EC, Adedokun OM, et al. Antiplasmodial activity of the n-hexane extract from *Pleurotus ostreatus* (Jacq. Ex. Fr) P. Kumm. Turkish J Phar Sci 2019;16(1):37–42.
7. Afieroho OE, Siwe Noundou X, Krause RWM, Isaacs M, Olley L, Hoppe HC, et al. An antiplasmodial depside from a Nigerian lichen *Dirinaria picta* epiphytic on the oil palm tree *Elaeis guineense.* Revista Boliviana De Quimica 2018;35(1):31–9.
8. Afieroho OE, Chukwu EC, Festus OH, Onyia CP, Suleiman M, Adedokun OM. Evaluation of the anti-mitotic and bacteriostatic activities of the fruiting bodies of *Pleurotus ostreatus* (Jacq. Ex. Fr) P. Kumm. (Pleurotaceae). Malaysian J Med Biol Sci 2017;4(1):15–20.
9. Afieroho OE, Ugoeze KC. Gas chromatography-mass spectroscopic (GC-MS) analysis of n-hexane extract of *Lentinus tuber-regium* (Fr) Fr (Polyporaceae) Syn *Pleurotus tuber regium* Fr sclerotia. Trop J Pharm Res 2014;13(11):1911–5.
10. Afieroho OE, Ollornwi KV, Elechi N, Okwubie L, Okoroafor D, Abo KA. Free radical scavenging potentials and level of some heavy metals in *Pleurotus flabellatus* Berk and Broome (Pleurotaceae). Global J Pharm Res 2013a;2(3):1807–12.
11. Afieroho OE, Lawson L, Olutayo MA, Emenyonu N. Antituberculosis and phytochemical investigation of the dichloromethane extract of *Pleurotus tuber-regium* (Fries) Singer sclerotium. Int Res J Pharm 2013b;4(1):255–7.
12. Houghton PJ, Raman A. Laboratory handbook for the fractionation of natural extracts. Chapman and Hall, London, UK, 1999.
13. Harborne JB. Phytochemical methods-a guide to modern techniques of plant analysis. 3rd edition, Chapman and Hall, London, UK, p 302, 1998.
14. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 1995;28:25–30.
15. Dinis TC, Madeira VM, Almeida ML. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophy 1994;315:161–9.

16. Gil-Ramire A, Pav-Caballero C, Baeza E, Nieves B, Gareia-Viguera C, Francisco R, et al. Mushroom do not contain flavonoids. J Funct Foods 2016;25:1–13.
17. Poggiali E, Cassinerio E, Zanaboni L, Cappellini MD. An update on iron chelation therapy. Blood Transfus 2012;10(4):411–22.
18. Martinez M, Alvarez ST, Campi MG, Bravo JA, Vila JL. Ergosterol from the mushroom *Laetiporus sp*.: isolation and structural characterization. Revista Boliviana De Quimica 2015;32(4):90–4.
19. Moreno A, Heleno SA, Barros L, Barreiro MF, Ferreira IC. Antioxidant activity of *Agaricus bisporus L*. hexane and ethanol extracts obtained by Soxhlet and ultrasound-assisted extraction: the importance of the presence of ergosterol. In 2nd Symposium on Medicinal Chemistry of University of Minho, 2015. Available via https://bibliotecadigital.ipb.pt/handle/10198/12380 (Accessed 29 May 2018).
20. Makropoulou M, Aligiannis N, Gonou Z, Pratsinis H, Skaltsounis AL, Fokialakis N. Antioxidant and cytotoxic activity of the wild edible mushroom *Gomphus clavatus*. J Med Food 2011;15(2):216–21.
21. Kageyama-Yahara N, Wang P, Wang X, Yamamoto T, Kadowaki M. The inhibitory effect of ergosterol, a bioactive constituent of a traditional Japaneseherbal medicine saireito on the activity of mucosal-type mast cells. Biol Pharm Bull 2010;33(1):142–5.
22. Vieira AT, Pinho V, Lepsch LB, Scavone C, Ribeiro IM, Tomassini T, et al. Mechanisms of the anti-inflammatory effects of the natural secosteroids physalins in a model of intestinal ischaemia and reperfusion injury. Br J Pharmacol 2005;146(2):244–51.

How to cite this article:
Okonkwo BO, Afieroho OE, Ahanonu ED, Okwubie L, Abo KA. *Pleurotus pulmonarius* (Fr.) Quel. (Pleurotaceae): *In vitro* anti-oxidant evaluation and the isolation of a steroidal isoprenoid. J Appl Biol Biotech 2019;7(04):32–38. DOI: 10.7324/JABB.2019.70406