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A Sesquiterpene Enol Carboxylic Acid from *Pleurotus tuber regium* Sclerotium

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Abstract

As a follow-up to an earlier report on the antihyperlipidaemic activity guided fractionation study on the chloroform extract from the sclerotia of the edible mushroom *Pleurotus tuber regium*, this present study is reporting the isolation of a sesquiterpene enol carboxylic from the active chromatography fraction of the chloroform extract of this edible mushroom. The purity of the compound was confirmed from TLC and Melting point determination. The chemical structure was elucidated using spectroscopic techniques (Mass spectrometry, 1&2D Nuclear magnetic resonance and Infra red spectroscopy). The isolated compound coded as compound P, exhibited molecular mass of 256 with molecular formular of $C_{14}H_{24}O_4$ and from the 1 &2D NMR and IR analyses, the compound P was identified as a sesquiterpene enol carboxylic acid with a sytemic name of 2,3-dihydroxy-10-propylcyclodec-2-ene-1-carboxylic acid.

Keywords: *Pleurotus tuber regium*, hyperlipidemia, sesquiterpene enol carboxylic acid.

INTRODUCTION

Hyperlipidemia can be defined as a state containing unusual high levels of lipids, elevated serum levels, mainly cholesterol and triglycerides in the blood (Omede *et al*., 2018). It is the main risk factor connected with cardiovascular diseases. Ischemic cerebrovascular disease and other related coronary hearts diseases are the major cause of death in the world according to the World Health Organization (Nag & Gush, 2013; WHO, 1994). Epidemiological evidence strongly sustained the positive correlation between hyperlipidemia, blood lipids, and its complications. The diseases linked up to unusual lipid metabolism are hypertension, diabetes, hyperlipidemia, and obesity (Verma, 2017). The blood lipids block the supply of blood to the heart through a deposit in the interstitial space of arteries known as atherosclerosis (Jijith and Jayakumari, 2018). The fat-protein complexes are best-known as lipoproteins, and they are either low-density lipoprotein (LDL), or high density lipoprotein (HDL). Hyperlipidemia is marked by the increment of very low density lipoprotein (VLDL), chylomicrons, and LDL and diminished HDL (Boekholdt *et al*., 2014; Velagapudi, 2017). Hyperlipdaemia therapy is aimed at reduction in the elevated LDL, triglyceride (TG) and total cholesterol (TC), alongside an increment in the diminished HDL values (Owolabi *et al*., 2013). Bile acid sequestrants, fibrates and statins which are the main lipid lowering drugs control the lipid metabolism by several mechanisms, though they have side effects such as flushing, abnormal liver function, diarrhea, muscle damage and nausea (Sagar *et al*., 2012).

Mushrooms from Pleurotaceae family are ingested and valued by man for their beautiful flavour, economical, ecological importance and medicinal possessions for some years (Beluhan and Ranogajec, 2011). They serve as nutraceuticals which furnishes health interests (Rathee *et al*., 2012). Their nutritional values are compared to those of meat, milk and egg. They are source of vitamins E and D, volatile oils and essential amino acid which contributes to our diet (Patel and Guya, 2012). Mushrooms are found effective against oxidative stress, asthma, reduction of hypercholesteromia, cancer and insomnia, in addition to exhibiting several other pharmacological activities (Singh, 2017). *Pleurotus tuber-regium* is a consumable mushroom belonging to the family Pleurotaceae and division of Basidiomycete. It is a tuberous wild species of white rot mushroom which produces fruiting bodies

from a unique globose sclerotium (Apetorgbor *et al*., 2013). *Pleurotus tuber-regium* has been used for the treatment of some ailments such as cold, fever, stomach ailments, asthma, small pox, high blood pressure, headache, malnourishment and also weight gain (Afieroho *et al*., 2013). As a follow-up to an earlier report on the antihyperlipidaemic activity guided fractionation study on the chloroform extract from the sclerotia of the edible mushroom *Pleurotus tuber regium* (Ezea *et al*, 2021a; 2021b), this report presents the isolation and characterisation of the chemical constituents of the active fractions.

MATERIALS AND METHODS

Sample Collection

Pleurotus tuber reguim sclerotia was purchased from Oil Mill Market, Port-Harcourt, Rivers State, Southern Nigeria and authenticated by a Mycologist at the International Centre for Ethnomedicine and Drug Development, Nsukka, Nigeria with Voucher specimen number: Inter CEDD/971.

Chemicals/Reagents/Equipments

Analytical grade chemicals (Merck, Germany) which includes absolute methanol, N-hexane and chloroform were used. Others include: distilled water, Glass Column, UV, TLC plates, NMR spectrometer, Infra-red spectrophotometer and mass spectrometer.

Extraction, Isolation, purification and characterization of compound P

The chloroform extract was obtained by exhaustive cold maceration as earlier reported (Ezea *et al.*, 2021a) and fractionated on a chromatography column(silica gel G, 200 -400 mesh-stationary phase) eluting with a gradient mixtures of n-hexane: chloroform: methanol (4:0:0; 3:1:0; 1:3:0; 0:1:0; 0:3:1; 0:2:2; 0:1:3; 0:4- v/v/v) and fractions with similar chromatography fingerprint on thin layer chromatography examination were pooled to afford four pooled chromatography fractions (F1, F2, F3 and F4) as reported earlier (Ezea *et al.*, 2021b). The most active fraction (F1) reported in the previous study (Ezea *et al.*, 2021b) was further separated on a glass column (1 x 55cm) parked with silica Gel G (200 – 400 mesh). The parked column was eluted with gradient mixtures of n-hexane, chloroform and methanol starting with 100% n-hexane before proceeding to n-hexane : chloroform (80:20, 60:40, 50:50, 40:60 and 20:80). The eluates were collected in 15 ml and spotted on analytical pre-coated TLC plates (silica gel GF_{254}) and developed in chloroform. Fractions with similar R_f values and colour reaction in daylight and UV before and after spray with 70% sulphuric acid were pooled to give 4 fractions (P1, P2, P3 & P4). The second fraction (P2), was further purified in a finger glass column 0.8 cm × 55 cm) with silica Gel G (200 – 400 mesh) using chloroform as the mobile phase while using analytical TLC as described above to track the elution of compound P as a blue fluorescent with purity confirmed as a single spot under UV (R_f of 0.79, Mobile phase: Chloroform,; Adsorbent: silical gel GF_{254}).

RESULTS AND DISCUSSION

Fraction P2 was purified using a glass finger column to produce compound P a white coloured crystal. It had an R_f value of 0.79 (Silica Gel, 0.25 mm, n-hexane: ethyl acetate- 1: 1) and fluoresced blue colour under UV lamp at 365 nm. The structure of this compound was elucidated by using IR, NMR (1-D and 2-D experiments) spectroscopy and Mass spectrometry.

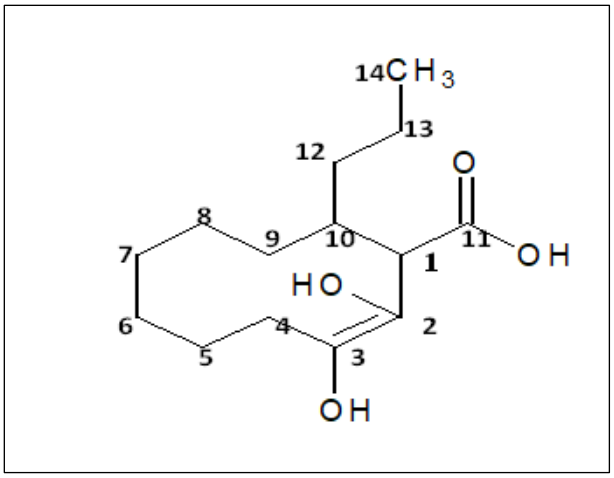

Figure 1: Chemical structure of Compound P

The IR gave information on major functional groups and elements that were present in compound P. As shown in Table 1, the IR bands were in the regions; 2954.53, 2915.1, and 2847.80 cm^{-1} representing CH asymmetrical of CH_3, CH asymmetrical of CH_2 and C-H symmetrical respectively. Also evident were the carbonyl stretch at 1700.63 cm^{-1}, and C-C deformation stretching vibrations at 1462.65 and 1430.95 cm^{-1}. There was a presence of C-O stretching due to the C-OH of the carboxylic acid. Also evident was the trans C=C deformation at 936.97 cm^{-1}. More bands were in the regions; 727.57, 720.07, and 686.96 cm^{-1} representing C-H rocking deformation of long chain $(CH_2)_n$ aliphatic. The structure of the compound P was established by ^{1}H-NMR, ^{13}C- NMR, ^{1}H-^{1}H COSY, DEPT -135, HSQC, and HMBC data (See Table 2). The ^{1}H-^{1}H -COSY spectrum revealed the correlation of the proton peaks and showed cross-peak correlations as in; proton H- 14 with H-13, and H-13 with H-14. One methyl proton triplet was evident at δ_H (ppm); H-14-Me (0.8). Also present were eight methylene (CH_2) protons corresponding to H-4, H–5, H-6, H-7, H-8, H-9, H-12 and H-13 respectively. Evident too were two methine (CH) protons at; H-1 and H-10 respectively. The unambiguous designations and identity of the chemical shift positions were achieved by the use of 2-Dimensional proton to carbon correlation (HMBC and HSQC). The other proton chemical shift peaks were equally rationalized on the same. A total of fourteen

carbon signals were identified in ^{13}C-NMR experiment of compound P. These were rationalized by the aid of DEPT-135 to be:. One methyl (CH_3) group at δc of 14ppm corresponding to C-14. Eight methylene (-$CH_{2)}$ groups at δc (ppm); 33.94 (C-4), 29.70 (C-5), 29.45 (C-6), 29.38 (C-7), 29.61 (C-8), 31.9 (C-9), 24.70 (C-12) and 22.71 (C-13). Evident also were two methine groups (-CH-) at δc (ppm); 31.45 (C-1) and 30.20 (C-10) respectively. Present from the 2D HMBC spectrum were two deshielded olefinic carbon cross peaks at 148 ppm (C-2) and 140 ppm (C-3). Evident also was one carboxylic group (COOH) at δc (ppm); 179.38 (C-11). Moreover, other correlations were evident in HMBC as rationalized in Table 2 below. The number assignment of hydrogen, carbon and oxygen was further supported in the MS spectrum with a molecular ion at m/z = 256 pm and equivalent to molecular mass of 256 ($C_{14}H_{24}O_4$), and diagnostic fragmentation peaks: [M-43] at m/z= 213 due to loss of the n-propyl group. The mass and NMR (ID and 2D) spectral data suggested the presence of sesquiterpene enol carboxylic acid derivative. Compound P is therefore a Sesquiterpene enol carboxylic acid derivative with (IUPAC) name as; (2,3-dihydroxy-10-propylcyclodec-2-ene-1-carboxylic acid. Several sesquiterpenoids isolated from the medicinal flora have been reported to exhibit antihyperlipidaemic activity. Some of such reported in literature include: cynaropicrin, aguerin B and grosheimin from the methanol leaves extract of *Cynara scolymus* L. (Shimoda *et al*., 2003), hanphyllin (Serino *et al.*, 2021), and several others belonging to the pseudoguanolides and germacranolides groups of sesquiterpenoids (Hall *et al.*, 1980).

Table 1: Interpretation of Infrared Spectrum of Compound P

S/No	Absorption band frequency (cm^{-1})	Nature of band	Description of band
1	2954.63	Sharp	CH asymmetric of CH_3
2	2915.11	Sharp	CH asymmetric of CH_2
3	2847.80	Weak	C-H symmetric
4	1700.63	Sharp	C=O stretching of COOH
5	1462.65, 1430.95	Weak	C-C deformation
6	1292.71, 1249.52, 1207.42	Weak	O-H bending
7	1186.58	Weak	C-O stretching of COOH
8	936.97	Weak	Axial C-OH
9	727.57, 720.07, 686.96	Weak	CH_2 rocking

Table 2: Nuclear Magnetic Resonance spectra data for Compound P

S/N	δC	δH	DEPT-135	H-H COSY	HMBC
1	31.45	1.4	CH		C_3
2	148	-	=C(OH)		
3	140	-	=C(OH)		
4	33.94	2.3	CH_2		C_3
5	29.70	1.3	CH_2		C_2, C_3
6	29.45	1.3	CH_2		C_3
7	29.38	1.3	CH_2		
8	29.61	1.3	CH_2		
9	31.9	1.4	CH_2		
10	30.20	1.3	CH		
11	179.38		COOH		
12	24.70	1.6,2.3	CH_2		C_{11}, $C_{10,}$ C_{13}, C_9
13	22.71	0.9,1.4	CH_2	H_{14}	
14	14.13	0.8	CH_3	H_{13}	C_{12}, C_{10}

CONCLUSION

The compound P isolated from *Pleurotus tuber reguim* was found to be a sesquiterpene enol carboxylic acid with a molecular mass of 256 and a molecular formular of $C_{14}H_{24}O_4$ characterized as 2,3-dihydroxy-10-propylcyclodec-2-ene-1-carboxylic acid. Further investigation is on-going to confirm its efficacy as a drug lead compound for the development of anti-hyperlipidaemic drugs.

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Conflict of Interest: As regards this work, the authors declare a no conflict of interest.

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