

## Antihyperlipidemic Activity and Phytochemical Screening of Chromatography Fractions from the Chloroform Extract of the Sclerotia of the Edible Mushroom *Pleurotus tuber reguim*

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### Abstract

*Pleurotus tuber reguim* is a mushroom widely used as food, and in traditional medicine for weight loss and treatment of headache, fever and stomach pain among other ailments. This present study is aimed at the evaluation of antihyperlipidemic activities of chromatography fractions obtained from an earlier reported anti-hyperlipidemic chloroform extract of *Pleurotus tuber reguim* using triton X-100 induced hyperlipidemic rat model as well as investigate the phytochemical constituent(s) from the active chromatography fraction(s). The pulverized sclerotia sample was extracted exhaustively by cold maceration with chloroform and the chloroform extract (6.42g) 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). Fractions with similar chromatography fingerprint on thin layer chromatography examination were pooled. Phytochemical screening was done using standard methods. The chloroform extract afforded four pooled chromatography fractions (F1, F2, F3 and F4). The trend: Total cholesterol [atorvastatin (47.5%) > F1 (42.3%) > F2 (37.6%) > F4 (29.9%) > F3 (29.8%)]; Triglyceride [F4 (47.6%) > F1 (44.8%) > Atorvastatin (35.6%) > F3 (32.9%) > F2 (4.3%)]; High density lipoprotein-cholesterol [F2 (101.3%) > F1 (91.2%) > atorvastatin (53.7%) > F3 (17.7%) > F4 (14.9%)]; Very low density lipoprotein-cholesterol [F4 (47.6%) > F1 (44.8%) > Atorvastatin (35.7%) > F3 (32.9%) > F2 (4.3%)]; Low density lipoprotein-cholesterol [Atorvastatin (12.5%) > F2 (10.0%) > F1 (7.6%) > F3 (3.5%) > F4 (-4.9%)]. Fractions F1 and F2 where found to contain triterpenoids and fatty acids while fractions F3 and F4 were found to contained amino acids or peptide derivatives. *Pleurotus tuber reguim* exhibited hyperlipidemic activity and the presence of triterpenoids and amino acids or peptide derivatives could be responsible for the observed trend in antihyperlipidemic activity.

**Keywords:** *Pleurotus tuber reguim*, Pleurotaceae, antihyperlipidemic, terpenoids, peptides.

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### INTRODUCTION

Hyperlipidemia helps significantly in the increase of blood lipoproteins. Cardiovascular diseases such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease are caused majorly by hyperlipidemia [1, 2]. Atherosclerosis and related coronary heart diseases are among the most common causes of mortality and morbidity worldwide. Approximately 12million people reportedly die of cardiovascular disease each year worldwide [3]. Treatment of hyperlipidaemia is geared towards a reduction in the elevated LDL, triglyceride (TG) and total cholesterol (TC), alongside an enhancement in the diminished HDL values since

hyperlipidemia is usually an elevated level of TC,TG and LDL, and decreased level of HDL [4, 5]. The known lipid-lowering drugs (fibrates, statins, bile acid sequestrants, etc.) regulate the lipid metabolism by different mechanisms, but they also have many side effects like hyper-uricemia, diarrhea, nausea, severe muscle damage (myopathy),gastric irritation, flushing, dry skin and abnormal liver function[6]. *Pleurotus tuber reguim* (Fr.) Sing is an edible mushroom found in tropical and sub-tropical regions of the world[7]. It is popularly consumed by the peoples of all over the world due to their high nutritional values and some medicinal properties. The *Pleurotus* mushrooms are rich in proteins, essential amino acids, Polysaccharides and essential fatty acids, dietary fibres, minerals, some

vitamins etc. [8]. Several pharmacological properties have been reported for extracts of *Pleurotus species* such as antihyperlipidemic activity[2, 9], antigenotoxic, bioantimutagenic[10], anti-inflammatory, antihypertensive, and antihyperglycaemic [9], Immunomodulatory[11], antibacterial and antifungal [12] activities. The folklore use of *Pleurotus tuber-regium* in Nigeria against weight loss reduction has been reported [13]. As a follow-up to an earlier report on the antihyperlipidemic properties of the chloroform extract of extract of *Pleurotus tuber reguum*, this report presents the antihyperlipidemic activities of chromatography fractions obtained from an earlier reported anti-hyperlipidemic chloroform extract of *Pleurotus tuber reguum* as well as investigation of the phytochemical constituent(s) from the active chromatography fraction(s).

## MATERIALS AND METHODS

### Sample collection

*Pleurotus tuber reguum* 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/Drugs/Reagents

Analytical grade chemicals (Merck, Germany) which includes absolute methanol, N-hexane and chloroform were used. Others include: Triton X-100 (Sigma-Aldrich, Germany), Atorvastatin, distilled water and Glass Column.

### Extraction and chromatography separation

The Chloroform (CHE) extract of *Pleurotus tuber regium* was obtained by cold maceration as earlier reported [2]. Briefly, the sample (10 kg) was cold extracted by soaking in chloroform as solvent for three consecutive days with agitation at interval of 6hrs and fresh replacement of solvent every 24hrs. The combined chloroform filtrate was, after concentration using a rotary evaporator and drying, kept as the chloroform extract CHE. The chloroform extract (6.42 g) was then fractionated on a chromatography column(silica gel G, 200 -400 mesh-stationary phase) and eluted 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; 0:0:4- v/v/v).. The chloroform extract afforded four pooled fractions (F1, F2, F3 and F4) based on the retardation factors ( $R_f$ ) and colour reactions with chromogenic spray reagents(ninhydrin and 10% conc.  $H_2SO_4$  in alcohol) of component bands from thin layer chromatography examination. Phytochemical test for the presence of metabolites was done using standard phytochemical screening reagents [14, 15].

### Animals

Thirty five (35) Wistar rats 170 – 200g were used for the study. The animals were provided ad libitum access to tap water throughout the experimental duration and maintained in a 12 hours light-dark cycle under standard laboratory conditions ( $22 \pm 2 ^\circ C$ ). All experiments were approved by the Research Ethics Committee of the University of Port Harcourt, Nigeria (approval Ref No.: UPH/CEREMADREC/MM67/016).

### Induction of Hyperlipidemia

Acute hyperlipidemia was induced in animals by intraperitoneal administration of Triton X-100 (Tyloxapol, Sigma-Aldrich) to the rats at a dose of 150 mg/kg body weight. Induced rats were allowed to stabilize for 72 hours before commencing treatment.

### Antihyperlipidemic Assay

The pooled column chromatography fractions (F1 – F4) were evaluated for *in vivo* anti-hyperlipidemic activities using reported method [16] with modification as earlier reported [2]. The study was carried out in the animal house of Faculty of Pharmaceutical Sciences, University of Port Harcourt. Briefly, Thirty five Wistar rats were randomly divided into seven groups each having 5 animals each (A-G). Group A was not given any treatment (normal control group), while the other six groups (B-G) were treated with single dose triton X-100 (150 mg/kg bw, ip) to induce hyperlipidemia. After hyperlipidemia induction, group B received no other treatment (hyperlipidemic untreated control group), while group C received standard antilipidemic drug atorvastatin (10 mg/kgbw, orally, groups D to G were administered 50 mg/kgbw the chromatography fractions (F1 –F4) orally, respectively. The agents were administered daily for 7 days. On the 8th day after the treatment, blood samples were collected by retro-orbital puncture in dry EDTA bottles and the serum lipid levels; total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) were determined using standard methods with commercially available kits.

### Data Presentation and Statistical Analysis

All data were expressed as mean  $\pm$  SEM. One way analysis of variance (ANOVA) and student's t-test were used to analyze the data and test for significance between experimental and hyperlipidaemic untreated control groups (p values  $< 0.05$  were considered significant).

## RESULTS AND DISCUSSION

Generally the blood lipid parameters TC, TG, LDL and VLDL were elevated with HDL reduced after 72 hours Triton-X100 inductions in the hyperlipidemic untreated control group compared to the normal control

group (Table-1). Hyperlipidemic groups treated with Atorvastatin (10 mg/kg) and chromatography fractions (F1-F4) from the chloroform extract of *Pleurotus tuber regium* at 50 mg/kgbw showed on a general note, ameliorating effect on the elevated levels of TC, TG, LDL, and VLDL as well as the reduced levels of HDL compared to the hyperlipidemic untreated control group (Table-1).

#### **Ameliorating effect on Total Cholesterol (TC)-cholesterol**

There was a significant ( $p < 0.05$ ) decrease in TC level in all the test chromatography fractions (F1-F4), and atorvastatin treated groups compared to hyperlipidemic untreated control group. Although, the atorvastatin treated groupd showed a relatively higher percentage ameliorating effect, it was not significantly different ( $p > 0.05$ ) from the test pooled chromatography fractions (F1-F4) treated groups as well as when compared with the normal control group (Table-1). The trend in the rank for TC was observed to be: atovastatin (47.5%)>F1 (42.3%)>F2 (37.6%)>F4 (29.9%)>F3 (29.8%).

#### **Ameliorating effect on Total triglyceride (TG)**

There is a significant ( $p < 0.05$ ) reduction in TG level in the pooled chromatography fractions F1 and F4 and atorvastatin treated groups, while pooled chromatography fractions F2 and F3 showed no significant ( $P > 0.05$ ) difference when compared to the hyperlipidemic untreated control (Table 1). The rank order of ameliorative effect on TG elevation by the agents was: F4 (47.6%)>F1 (44.8%)>Atorvastatin (35.6%)>F3(32.9%)>F2(4.3%).

#### **Ameliorating effect on High Density Lipoproteins (HDL)-cholesterol**

The pooled chromatography fractions F2 and F1 increased (101.2 and 91.2 % respectively) HDL level more than the other pooled chromatography fractions and the standard drug Atorvastatin (53.7%). The observed ameliorating (elevating) effect on the HDL levels for the F1 and F2 treated group were significant ( $p < 0.05$ ) compared to the hyperlipideamic untreated group (Table-1). The general trend in the calculated ameliorating effect for HDL-cholesterol was observed to be: F2(101.3%)>F1(91.2%) >atovastatin (53.7%)>F3(17.7%)>F4(14.9%). The effect of HDL is to make the transportation of cholesterol easy from peripheral tissues such as arteries to the liver for catabolism [17]. This results in the reduction of cholesterol and triglycerides and hence a reduction in cardiovascular risk. HDL has been recognised as the protective cholesterol fraction (good cholesterol) because there is an inverse relationship between the

concentration of HDL- cholesterol and the agent of cardiovascular complications.

#### **Ameliorating effect on Very Low Density Lipoproteins (VLDL)- cholesterol**

VLDL is a proportion of the TG and its plasma elevation just like TC and TG, is a risk factor in the progression of arteriosclerosis, and related cardiovascular diseases. In this investigation, the elevated VLDL cholesterol peaked in the hyperlipidemic untreated controls group and for the hyperlipidemic groups treated with the various pooled chromatography fractions (F1-F4) and atorvastatin, there was a significant ( $p < 0.05$ ) ameliorative effect (reduction in VLDL levels) observed for the groups treated with F1, F4 and atorvastatin. The ranked order in amelioration of the elevated VLDL-cholesterol was observed to be: F4 (47.6%)>F1(44.8%)>Atorvastatin(35.7%)>F3(32.9%)>F2(4.3%).

#### **Ameliorating effect on Low Density Lipoproteins (LDL)-cholesterol**

Elevated LDL- cholesterol is a risk to cardiovascular health, unlike HDL-cholesterol which is good cholesterol. From the result, the elevation of LDL-cholesterol seen in the hyperlipidemic untreated group were reduced except for the group treated chromatography fraction F4 (-4.9%) although not significantly ( $p > 0.05$ ). The general trend in the calculated ameliotating effect for LDL- cholesterol was observed to be: Atorvastatin (12.5%) > F2 (10.0%)>F1 (7.6%)>F3(3.5%)>F4(-4.9%).

#### **Phytochemical screening results**

Triterpenoids and fatty acids were detected in the chromatography fractions F1 and F2 while as observed for the ninhydrin test, peptide bonds containing metabolites were detected in chromatography fractions F3 and F4 as seen in Table 2 and Figure 1. In an earlier phytochemical study [18], ergosterol and poly-unsaturated fatty acids derivatives have been characterized from this mushroom using Gas-Chromatography-Mass Spectrometry techniques. Ergosterol derivatives and related phytosterols have been reported to be antihyperlipidemic [9, 19, 20]. Poly unsaturated fatty acids such as the omega-3 derivatives are also good in the management of obesity and cardiovascular ailments unlike the saturated fatty acids. Essential amino acids like methionone, threonine and tryptophan have also been found to reduce cholesterol level and prevent fatty liver [21]. The observed anti-hyperlpidemic activity of the extracts from the sclerotia of this edible mushroom *P. tuber regium* could therefore be attributed to the presence of one or more of these phytochemicals as detected in Table 2.

**Table-1: Antihyperlipidemic activity of the chromatography fractions (F1-F4) from the chloroform extract of *Pleurotus tuber regium*.**

| Blood lipid parameters | Normal control           | Hyperlipidemic untreated | F1                                | F2                                 | F3                               | F4                               | Atorvastatin 10 mg/kgbw          |
|------------------------|--------------------------|--------------------------|-----------------------------------|------------------------------------|----------------------------------|----------------------------------|----------------------------------|
| TC                     | 1.044±0.019 <sup>b</sup> | 2.108±0.106              | 1.216±0.093 (42.3%) <sup>b</sup>  | 1.315±0.128 (37.6%) <sup>b</sup>   | 1.480±0.162 (29.8%) <sup>b</sup> | 1.478±0.126 (29.9%) <sup>b</sup> | 1.106±0.157 (47.5%) <sup>b</sup> |
| TG                     | 0.726±0.126              | 1.050±0.135              | 0.580±0.075 (44.8%) <sup>b</sup>  | 1.005±0.161 (4.3%)                 | 0.705±0.126 (32.9%)              | 0.550±0.137 (47.6%) <sup>b</sup> | 0.676±0.040 (35.6%) <sup>b</sup> |
| HDL                    | 0.648±0.060 <sup>b</sup> | 0.272±0.088              | 0.520±0.023 (-91.2%) <sup>b</sup> | 0.548±0.075 (-101.3%) <sup>b</sup> | 0.320±0.050 (-17.7%)             | 0.313±0.029 (-14.9%)             | 0.418±0.058 (-53.7%)             |
| LDL                    | 0.382±0.055 <sup>b</sup> | 0.658±0.046              | 0.608±0.071 (7.6%)                | 0.593±0.102 (10.0%)                | 0.635±0.059 (3.5%)               | 0.690±0.066 (-4.9%)              | 0.576±0.056 (12.5%)              |
| VLDL                   | 0.145±0.025              | 0.210±0.027              | 0.116±0.015 (44.8%) <sup>b</sup>  | 0.201±0.032 (4.3%)                 | 0.141±0.025 (32.9%)              | 0.110±0.028 (47.6%) <sup>b</sup> | 0.135±0.008 (35.7%) <sup>b</sup> |

Values are mean ± SEM; <sup>b</sup>significantly different from the hyperlipideamic untreated group

**Table-2: Phytochemical constituents detected in the chromatography fractions (F1-F4) from the chloroform extract of *Pleurotus tuber regium*.**

| Plant metabolites                     | F1      | F2      | F3      | F4      |
|---------------------------------------|---------|---------|---------|---------|
| Triterpenoids(Lieberman-Buchard test) | present | present | absent  | absent  |
| Peptide derivatives(Ninhydrin test)   | absent  | absent  | present | present |
| Fatty acids                           | present | present | absent  | absent  |

## CONCLUSIONS

This work demonstrated that the chromatography fractions from the chloroform extract of *Pleurotus tuber-regium* possess lipid lowering effect which suggests that the sclerotium has anti-hyperlipidemic properties. The class of chemical constituents (triterpenoids and peptides bonds containing derivatives) could be responsible for the varying degree of ameliorating effects on the various blood lipid parameters observed. Further investigation is on-going to purified and elucidate the chemical structure of the pure metabolites and evaluate them for anti-hyperlipidemic activity which could serve as leads in the development of novel and useful drugs for the management of obesity and related complications due to diabetes and cardiovascular ailments.

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