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Antiplasmodial activity of the lipophilic and hydrophillic fractions of *Pleurotus ostreatus* (Jacq. Ex. Fr) P. Kumm. on *Plasmodium berghei* infected mice model

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Abstract

Introduction: As a follow-up to our earlier report on the *in vitro* plasmodium lactate dehydrogenase (pLDH) inhibition activity of the edible mushroom *Pleurotus ostreatus*, this present study is reporting the *in vivo* anti-plasmodial activity of the lipophilic and hydrophilic fractions from this edible mushroom.

Methodology: Freshly collected *P. ostreatus* (5 kg) was cold macerated in absolute ethanol for 72 hours. The ethanol extract was concentrated *in vacuo* to one-tenth and partitioned with dichloromethane giving the lipophilic LF (dichloromethane) and hydrophilic HF (aqueous) fractions. Lorke's method was used for the acute toxicity evaluation of LF and HF. Anti-plasmodial assay was done *in vivo* using the Rane's four

days suppressive model on chloroquine sensitive *Plasmodium berghei* (ANKA strain) infected mice with chloroquine as standard control drug. Phytochemical analysis was by using standard methods. Student's t-test of significance ($p < 0.05$) was used for data analysis**.**

Results: The LF (yield 0.46 % w/w ; LD_{50} > 5000 mg/kg bw; supressive activity (10 and 1000 mg/kg bw): 71.69 % and 70.91 %) and HF (yield 0.41 % w/w; LD_{50} > 5000 mg/kg bw; supressive activity (10 and 1000 mg/kg bw): 69.3 % and 70.4 %) were both non-toxic and significantly ($p < 0.5$) suppresive *in vivo* compared to the untreated group though not as active when compared to chloroquine (100 % suppresion). Mice treated with the higher dose of the HF, showed lesser weight reduction and higher survival rate compared to the lower dose. Phytochemicals: (LF:Fixed oils and triterpenoids and HF :amino acids, and carbohydrates) were present with saponins, phenolics compounds, cyanogenic glycosides, anthraquinones and alkaloids absent in both fractions.

Conclusion: This observed *in vivo* antiplasmodial activity corroborated the earlier reported *in vitro* pLDH activity of this edible mushroom and is a further evidence of its nutraceutical potential in the management of malaria.

Keywords: *Pleurotus ostreatus*, nutraceuticals, malaria, triterpenoids, amino acids,

carbohydrates

INTRODUCTION

The global burden of malaria infections and associated complications have continued to be a challenge for countries in Sub-Saharan Africa. Mostly affected are the poor people, children and expectant mothers in endemic countries with associated high mortality if not treated promptly[1]. Drug resistant *Plasmodium falciparum* strains of the causative parasite in addition to limited access to quality health care facilities and the high cost of orthodox drugs are obstacles necessitating the need for a continued search for new and relatively non-toxic anti-malarial agents.. Edible mushrooms are patronized for their nutritive value and functional uses. The anti-malarial [2-3], amoebicidal[4] anti-trypanosomiasis[5] nematicidal[6], anti-inflammatory and immunomodulatory[7] and anticancer[8-9] properties of some edible mushrooms have been documented. As a follow up to earlier reports on the *in vitro* anti-plasmodial activity of *Pleurotus ostreatus*[10] a.bioactive chemical entities bioprospection and nutraceutical potentials of edible Nigerian mushrooms and related mycoflora[11-14] ,this present study is aimed at the evaluation of the *in vivo* anti-plasmodial activity of *Pleurotus ostreatus* using *Plasmodium berghei* infected experimental mice model.

Materials and Methods

Collection of mushroom sample

Pleurotus ostreatus (Fresh fruiting bodies) were collected from the Dilomat farm, Rivers State University of Science and Technology (RSUST), Port Harcourt, Rivers State and

identified by a Mycologist in the Department of Crop and Soil Sciences, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Rivers State. After due authentication, a voucher specimen (UPH/C/075) was deposited at the herbarium of the Department of Plant Science and Biotechnology of the same University. The fresh fruiting bodies of *Pleurotus ostreatus* were chopped into small pieces after which they were dried under a current of air in a de-humidified environment. The dried samples were pulverized using an electric blender.

Experimental Animals

A total of sixty-six experimental albino mice (25±3g) of both sexes purchased from the animal house of the Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria were used for this study. Due animal handling and ethics guideline were followed. The animals were kept and acclimatized for two weeks in cages, maintained at room temperature under 12 hours dark and light cycle in the animal house and fed with standard diet and portable water *ad libitum*.

Preparation of extract

The freshly collected fruiting body (5 kg) was quenched and cold macerated for 72 hours in absolute ethanol with fresh replacement of solvent at 24 hours interval to obtain the ethanol extract which was concentrated to about one-tenth its volume using a rotary evaporator and subsequently partitioned with dichloromethane to obtain the lipophillic (dichloromethane) fraction (LF) and the

polar (aqueous) fraction (HF) used for this study.

Acute Toxicity

This was conducted in a staircase method on experimental albino mice(25-28 g) as reported by Lorke[15] . Briefly, the mice were separately divided into six groups of three mice each according to their body weight. On the first day of the test, the mice in groups 1 to 3 were given 10, 100, and 1000 mg/kg body weight of the test lipophilic fraction LF by oral route using oral gavages to ensure safe ingestion of the preparation and observed for signs of toxicity. After twenty four hours, the remaining three groups were given 1600, 2900 and 5000 mg/kg body weight. The same procedure was done for the hydrophilic fraction HF.

***In vivo* antiplasmodial evaluation**

The Peters' 4-day suppressive test against *P. berghei* infected mice was employed with slight modification[16]. Briefly, adult Swiss albino mice weighing (25-28g) were divided into six groups (A-G) of five mice each per cage and were inoculated by intra-peritoneal (i.p.) injection with 0.1 ml of a diluted blood (in normal saline) containing 2 $x10^7$ parasitized erythrocytes. The mice were then treated for four (4) consecutive days with daily doses of the fractions, chloroquine or the vehicle by oral route as follows: Mice in groups A and B were respectively treated with 1000, and 10 mg/kg bw of LF, mice in groups C and D were respectively treated with 1000, and 10 mg/kg bw of HF, mice in

group E (standard drug control group) were treated with 10 mg/kg bw chloroquine, mice in F (DMSO negative control for LF) and group G (distilled water negative control for HF) were treated with 0.1 ml of DMSO and distilled water respectively.. On day five (5) of the test, thin blood smears were prepared and blood films were fixed with methanol and the blood films stained with Giemsa, followed by examination under the microscope(1000 x magnification). The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. Mortality was also monitored daily and throughout the period of study and the percentage survival time determined. Also monitored during the period of study was the change in body weight of each mouse using a sensitive electric weighing balance and the percentage change in body weight of the test samples treated groups compared to the control was determined. All data expressed as mean ± standard deviation, were analysis using the student's t-test of significance ($p < 0.05$)

Phytochemical screening:

This was done using standard phytochemical screening reagents[17-18]. Phytochemical constituents screened for include: alkaloids, phenolics, saponins, cyanogenic glycosides, anthraquinones, triterpenoids, carbohydrates, amino acids and fixed oils.

RESULTS

The yield of the LF and HF were 0.46 and 0.41 % w/w. Both exhibited an $LD_{50} > 5000$

mg/kg bw (see Table 1). Table 2 showed the result of the four day suppressive anti-plasmodial assay and the effect on body weight and survival using the experimental *P. berghei* infected mice model. Whereas fixed oils and triterpenoids were the only detected phytochemicals in the LF, only amino acids, and carbohydrates were observed for HF with saponins, flavonoids, cyanogenic glycosides, anthraquinones and alkaloids absent in both fractions (see Table 3).

DISCUSSION

The result of the acute toxicity in Table 1 showed that both the lipophillic (LF) and hydrophilic (HF) fractions from the edible mushroom *P. ostreatus* are non-toxic as neither death nor visible signs of toxicity was observed even at the highest dose of 5000 mg/kgbw. This implies LD_{50} of greater than 5000 mg/kg bw. This is an indication of the high safety margin of the constituents in these two fractions from this edible mushroom. Four days suppressive anti-malarial test is a preliminary test for the determination of anti-malarial activity in *P. berghei* infected mice. In this study, both the lipophilic LF and hydrophilic HF fractions treated groups showed significant ($p < 0.05$) percentage parasitaemia suppression effect compared to the untreated group though not as active when compared to chloroquine (100 % suppresion). Although this effect was not significantly($p > 0.05$) dose dependent it could be regarded as active. A substance is considered to be active when the percentage suppression of parasitaemia is greater than 30

% [19-20]. Unlike the LF, mice treated the HF (1000 mg/kg bw) were observed to have a significant (p , 0.05) lesser weight reduction effect compared to the group treated with the standard drug chloroquine (see Table 2). This could be attributed to the adverse effect of chloroquine like anaemia, hypoglyceamia and loss of appetite. Thus the constituents in HF could help in the amelioration of these possible fatal side effects associated with malaria. The presence of amino acids and carbohydrates as constituents in the HF could offer a rationale for this trend. Fungal polysaccharides[3]and proteins[21] have been reported to exhibit immunomodulatory effects. Immunostimulatory water soluble polysaccharides like the Lentinans from the mushroom *Bulgaria inguinans* have been investigated for anti-malarial activity[3]. Generally,mice treated with the higher dose of the LF or HF, showed higher survival rate compared to lower dose . The presence of the lipophillic constituents: fixed oils and triterpenoids in the LF, and hydrophilic constituents:amino acids, and carbohydrates in the HF could offer a rationale for the observed trend in activity.

CONCLUSION

This observed trend in anti-plasmodial activity *in vivo,* corroborated the earlier reported *in vitro* activity[10] and is a further evidence supporting the nutraceutical potential in the management of malaria infection, of the edible mushroom *Pleurotus ostreatus* cultivated in Nigeria.. After further investigation, this edible mushroom species

may be recommended in the diet as a prophylaxis against malaria infection.

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CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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Table 1: Result of acute toxicity evaluation of the lipophillic and polar fractions from *P.ostreatus* fruiting bodies against healthy mice model

Dose (mg/kg body weight)	Lipophillic fraction (LF)	Polar fraction (HF)
10	0/3	0/3
100	0/3	0/3
1000	0/3	0/3
1600	0/3	0/3
2900	0/3	0/3
5000	0/3	0/3
LD_{50}	>5000 mg/kg bw	>5000 mg/kg bw

Table 2: Result of anti-plasmodial (four days chemosuppressive) activity of the lipophillic and polar fractions from *P.ostreatus* fruiting bodies against *P. berghei* infected mice model

Test sample	Dose per kg body weight	% Chemosuppression	% weight loss	% survival rate
Lipophillic	1000 mg	70.91±0.72	26.10±3.05	40
fraction (LF)	10 mg	71.67±1.07	20.60±0.77	20
DMSO	10 ml	0.00	28.80±2.51	20
Polar	1000 mg	70.43± 8.33	3.95*±0.20	40
fraction (HF)	10 mg	69.35±0.00	22.90±8.83	20
Distilled water	10 ml	0.00	27.62±6.17	20
Chloroquine	10 mg	100.00	25.50±1.18	100

*Significant ($p < 0.05$compared to the standard drug chloroquine

Table 3: Results of the phytochemical screening of the lipophillic and polar fractions from *P.ostreatus* fruiting bodies

Phytochemical constituents)	Lipophillic fraction (LF)	Hydrophilic fraction (HF)
Alkaloids	Absent	Absent
Saponins	Absent	Absent
Phenolics	Absent	Absent
Anthraquinones	Absent	Absent
Cyanogenic glycosides	Absent	Absent
Triterpenoids	present	Absent
Amino acids	Absent	present
Carbohydrates	Absent	Present
Fixed oils	Present	Absent