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Evaluation of the Anti-mitotic and Bacteriostatic Activities of the Fruiting Bodies of Pleurotus Ostreatus (Jacq. Ex. Fr) P. Kumm. (Pleurotaceae)

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ABSTRACT

Edible mushrooms aside being taken as foods, are used in ethnomedicine in the management of various ailments notably tumours and related ailments whose pathophysiology are linked to oxidative stress. This study investigated the anti-mitotic, and antibacterial activities of the aqueous extract of the edible mushroom *Pleurotus ostreatus* based on ethno-medicine.The *Allium cepa* anti-mitotic assay model was used for anti-proliferative investigation of the defatted aqueous ethanol extract at concentration range of: 10.00 – 0.08 ng/mL following a two-fold serial dilution approach. Methotrexate (0.25 ng/mL) and portable water were used as reference standard for positive control and negative control respectively. The student t-test was used for statistical analysis ($p < 0.05$). Antibacterial susceptibility evaluation against clinical isolates of selected pathogenic organisms: *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aereus,* and *Bacillus subtilis* was done using the Agar dilution method at 1000 and 10 µg/mL. The aqueous extract showed a dose and time dependent anti-mitotic activity with the three higher doses: 10.00, 5.00 and 2.50 ng/mL exhibiting complete inhibition of mitosis which was comparable to the reference drug methotrexate (0.25 ng/mL) after 96 hours incubation period. Although the aqueous extract was not bacteriocidal at the test concentration, a dose dependent bacteriostatic effect against *E. coli,* and *B. subtilis* was observed. The observed anti-mitotic activity of this mushroom validates its ethnomedicinal use in the treatment of tumours and related diseases.

Key words: Mushroom, anti-proliferation, bacteriostatic, *Pleurotus ostreatus,* ethnomedicine

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INTRODUCTION

Cancer epidemic is on the rise. Current radio and chemotherapy regimen have suffered drawbacks due to non-selectivity and the associated fatal side effects. Aside tobacco usage, obesity, poor diet, lack of physical activity, ionising radiation, environmental pollutants, inherited genes and alcohol consumption which pre-dispose humans to cancer. Other factors like bacterial, viral and parasitic infections have also been linked to cancer. Also of concern is the high risk pre-disposition of cancer patients to opportunistic bacterial and fungal infections especially in severe neutropenia (Rolston, 2009). As a result of drawbacks in radio and chemotherapy, interest in the use of nutraceuticals and change in diet and lifestyle are being embraced in the management of cancer with clinical successes. This justifies the shift to nature armament of medicines for nutraceutical supplement of mostly herbal origin, as alternative treatment with lesser toxicity to non-cancerous cells.

The primary mechanism by which organisms generate new cells is through cell division. Mitosis is a method of cell division in which a cell divides and produces identical copies of itself. Cancerous cells are known to grow through rampant and unregulated cell divisions. Selective inhibition of mitosis therefore offers a good rationale for the

development of anticancer drug. The *Allium cepa* anti-mitotic assay monitors the degree of root meristemic cell growth inhibition in the presence of an anti-mitotic agent (Sanjib and Pallab 2012).

First cultivated as a subsistence measure during World War 1 (Edger *et al*, 1976), *P. ostreatus* like every other edible mushroom, is now being grown commercially around the world for food and nutraceutical purposes. The oyster mushrooms are known for their antitumour (Wandati *et al* 2013), antioxidant (Afieroho and Ollornwi *et al*, 2013), antibacterial properties (Vanamu et *al* 2012; Afieroho and Lawson *et al*, 2013), antidiabetic properties (Ghaly *et al* 2011), antiviral (Piraino and Brandt 1999) and nematophagous properties (Chang and Philip, 2004). As a follow up to earlier reports aimed at the characterisation of bioactive secondary metabolites from indigenous edible mushrooms in Nigeria (Afieroho and Ugoeze, 2014, Afieroho and Ollornwi *et. al* 2013, Afieroho and Lawson *et. al*, 2013), this present study is aimed at investigating the anti-mitotic, and antibacterial activities of the aqueous ethanol extract of the edible mushroom *Pleurotus ostreatus* with the view of validating its related ethnomedicinal uses.

MATERIALS AND METHODS

Plant Material

Fresh fruiting bodies of *Pleurotus ostreatus* used for this study were collected from the Dilomat Farm, Rivers State University of Science and Technology (RSUST), Port Harcourt, Rivers State, Nigeria in the month of August 2015, and authenticated by a Mycologist in the Department of Crop and Soil Sciences, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. A voucher specimen (UPH/C/075) has been deposited at the herbarium of the Department of Plant of Science and Biotechnology of the same University. The fresh fruiting bodies of *Pleurotus ostreatus* were chopped into small pieces after which they were air dried under a current of air in an ambient condition. The air dried samples were pulverized using an electric blender.

Preparation of defatted aqueous ethanol extract

The dried pulverised fruiting bodies were first cold macerated for 72 hours in n-hexane, and dichloromethane in succession to remove lipophilic or fatty constituents. The defatted residue after air drying (to remove residual solvents) was further extracted with 75 % aqueous ethanol for 72 hours with fresh replacement of solvent at 24 hours interval. The pooled aqueous ethanol extract was then concentrated using a rotary evaporator and the concentrated solution further partitioned with dichloromethane to remove residual lipophilic constituents to obtain the lipophillic/fatty constituents-free aqueous ethanol extract (AQE) used for this study.

Antimitotic Activity

This was done using the *Allium cepa* anti-mitotic assay model (Grant 1982, and Shweta *et al* 2007) with necessary modification. Approximately equal sized (32.3 g) bulbs of the onions *(Allium cepa* L.) were obtained from the local vegetable market at Choba, Rivers State. Onion bulbs that were dry, moldy or have started shooting green leaves were discarded. The chosen bulbs were grown in the dark for 48 hours in a 10 ml capacity beaker containing tap water at ambient temperature until the roots have grown to approximately 1-2 cm. The viable bulbs were then selected and used for subsequent studies. The bulbs with root tips greater than 1 cm were removed from the tap water and placed into different concentrations of the aqueous ethanol extract (10.00, 5.00, 2.50, 1.25, 0.63, 0.31, 0.16 and 0.08 ng/mL), negative control(tap water) and positive control (methotrexate: 0.25 ng/mL). The onions bulbs root tips were grown in the dark for 96 hours in a 10 ml capacity beaker at ambient temperature and the elongation was measured after every 24 hours. The root tips were harvested and placed in micro tubes, they were placed on a water bath for 12 minutes, and 0.1 N HCl was added to the micro tubes to fill two-third of the tubes. They were placed on a water bath for 12 minutes, after which the 0.1 N HCl was removed. The root tips were rinsed severally with distilled water and a small section was cut and placed on a microscope slide. The root tips were then flooded with acetocarmine stain, and the slide was rinsed with distilled water. The slide was covered with a cover slip, mounted on a compound microscope and viewed at x10 and x40 objectives, and also on a photo microscope to obtain the pictures of structural features observed. The results obtained were recorded.

Antibacterial susceptibility evaluation

This was done using the Agar dilution method with slight modification where necessary. A 0.1 McFarland inoculums of clinical isolates of selected pathogenic organisms: *E. coli, P. aeruginosa, K. pneumonia, S. aereus,* and *B. subtilis* were used respectively at two final test extract's concentration 1000 and 10 µg/mL after re-constitution in the Mueller Hinton agar. Briefly, a ten (10) fold dilution of a 10 mg/mL aqueous stock solution of the lipophillic constituents-free aqueous ethanol extract of the *P. ostreatus* was made in the Mueller Hinton agar and poured into a sterile petri dish. After 24 hours prior incubation at 37ºC, a 0.1 mL aliquot of the respective 0.1 McFarland inoculums of any of the selected pathogenic bacteria was inoculated. This gives a test concentration of 1 mg/mL (1000µg/mL). Similar tenfold dilutions were made using the 0.1 mg/mL aqueous stock solutions of the lipophillic constituents-free aqueous ethanol extract of the *P. ostreatus* respectively to obtain the second final test concentration of 0.01 mg/mL (10µg/mL).

Phytochemical Methods

Confirmatory phytochemical tests were carried out on the extract using the standard phytochemical screening reagents (Houghton and Raman, 1999).

RESULTS AND DISCUSSION

In the evaluation of the anti-mitotic activity of *P. ostreatus* fruiting bodies, using the *Allium cepa* model, the aqueous ethanol extract showed a dose-dependent inhibition of the meristematic cells elongation (Table 1). Higher concentrations of the extract (10.0, 5.0, 2.5 ng/mL) showed a complete inhibition of the root tips elongation comparable to the standard methotrexate (0.25 ng/ml). This indicated that at higher concentrations, the anti-mitotic index of the extract is similar to that of the reference drug methotrexate (0.25ng/mL). Even at the least concentration (0.078 ng/mL) the percentage inhibition were observed to be significantly higher ($p < 0.05$) compared to that of the control (tap water) after 96 hours of growth incubation. Further confirmatory evidence of genotoxicity activity was seen from the microscopic examination of the meristematic cells using acetocarmine stain (Fig. 1a-d). This showed the absence of dividing cells in the root tips as seen with the absence of stained chromatids at the highest concentration of the aqueous ethanol extract (10.0 ng/mL; see Fig. 1b) and also that of the reference drug methotrexate (0.25 ng/ml; see Fig. 1a). This is in contrast to the observed presence of dividing cells in the negative control (tap water, Fig. 1d) and also for the root tips at the lowest concentration of the extract (0.078 ng/mL; Fig. 1c) of exposure as seen with the presence of stained chromatids. The trend in the results is evident that the aqueous ethanol extract of *P. ostreatus* has anti-mitotic and genotoxic potentials which could be harnessed in the development of drugs and nutraceuticals for the treatment of tumours and related cancerous ailments. Mitosis is a method of cell division in which a cell divides and produces identical copies of itself. The primary mechanism by which organisms generate new cells is through cell division. Cancerous cells are known to proliferate through rampant and unregulated cell divisions. If mitosis occurs, it means that cells are actively dividing. Actively dividing cells may lose control (Mutation) and when this happens, cancer can set in (Grant 1982, and Shweta *et al* 2007, Sanjib and Pallab 2012).

Table1: Anti-mitotic activity of the aqueous ethanol extract of *Pleurotus ostreatus* using *Allium cepa* model

Concentration(ng/mL)	%Elongation at 24 hours	%Elongation at 48 hours	%Elongation at 72 hours	%Elongation at 96 hours
10.000	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5.000	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2.500	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1.250	1.6 ± 1.0	2.7 ± 1.0	5.6 ± 0.9	8.2* ± 0.8
0.625	2.3 ± 0.5	4.2 ± 0.7	5.5 ± 0.6	7.4* ± 0.7
0.313	2.1 ± 0.6	5.9 ± 0.7	7.9 ± 0.7	11.8* ± 0.7
0.156	1.9± 0.8	5.4 ± 1.0	8.0 ± 1.0	10.0* ± 0.6
0.078	1.6 ± 0.7	5.5 ± 0.4	8.7 ± 4.6	12.0* ± 0.6
Control (tap water)	3.7 ± 0.9	9.5 ± 0.9	14.4 ± 0.9	17.3±0.9
Methotrexate(0.250 ng/mL)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Note: values are expressed as mean ± standard deviation (n=3).
* denote significant growth inhibition ($p<0.05$) after 96 hours compared to control (tap water)

Figure 1a: Photomicrograph of *Allium cepa* root tip meristem after 96 hours exposure to methotrexate (reference drug/positive control) at 0.25 ng/mL showing absence of dividing cells

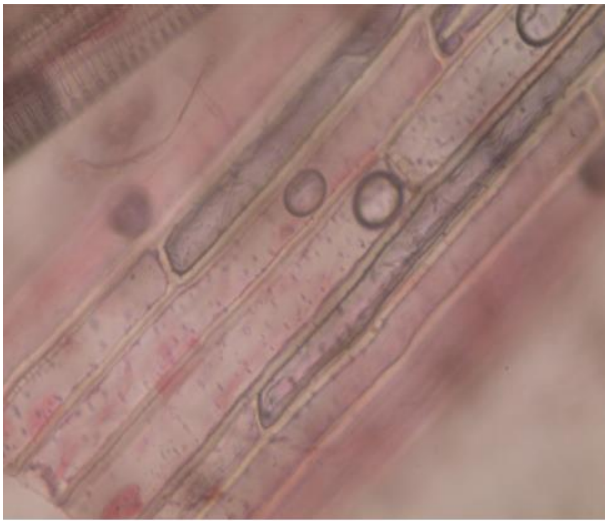

Figure 1b: Photomicrograph of *Allium cepa* root tip meristem after 96 hours exposure to aqueous ethanol extract of *P. ostreatus* at 10 ng/mL showing absence of dividing cells

Figure 1c: Photomicrograph of *Allium cepa* root tip meristem after 96 hours exposure to after 96 hours exposure to aqueous ethanol extract of *P. ostreatus* at 0.078 ng/mL showing less prominent dividing cells

Figure 1d: Photomicrograph of *Allium cepa* root tip meristem after 96 hours exposure to after 96 hours exposure to tap water (negative control) showing rapidly dividing cells

Table 2 showed the results from the antibacterial susceptibility study. After 24 hours of incubation, the aqueous ethanol extract showed activity against *E coli* and *B subtillis* only at the 1000 µg/ml (1mg/ml) test concentration. However, this observed inhibitory effect was not sustained after 48 hours incubation. This shows a bacteriostatic effect and not a bactericidal effect. Phytochemical screening of the aqueous ethanol extract revealed the presence of carbohydrates, cardiac glycosides, trace amount of alkaloids but absence of flavonoids and saponins. The trend in the results of the antibacterial assay for the Gram negative *E.coli* and the Gram positive *B.subtilis* is evident that the aqueous extract of *P. ostreatus* has bacteriostatic and not bacteriocidal potentials. Bacteriostatic agents are agents capable of suppressing the growth of bacteria without killing them out rightly, therefore they must work together with the immune system to remove the microorganism from the body. High concentrations of some bacteriostatic agents are bactericidal. Bactericidal agents on the other hand, are agents that kill bacteria without giving them room for growth irrespective of the condition whether favorable or not. However, the no growth inhibition observed against the other pathogenic bacteria at the final test concentrations of 1000 and 10 µg/mL respectively, may be attributed to strain resistance considering their being cultures from clinical samples. Tosun *et al* (2004) reported that for a crude plant extract to be considered active and promising in terms of leading drug development, it should inhibit the test pathogenic micro-organism at a concentration ≤ 200 µg/mL. Some other authorities consider crude extracts that inhibited the growth of the pathogenic micro-organism at a concentration ≤ 0.1mg/ml as significant, 0.1-0.625 mg/mL as moderate and >0.625 mg/mL (Eloff, 2004; Kuete 2010; Rios and Recios 2005; Awouafack *et al*, 2013). For isolated pure compounds, antimicrobial activity is said to be significant if the MIC is < 10 µg/mL, moderate if in the range 10- 100 µg/mL and low if > 100 µg/mL (Kuete 2010; Rios and Recios 2005). This justifies the choice of using 1000µg/mL (1mg/mL) and 10µg/mL (0.01mg/mL) for the anti-bacterial susceptibility screening in this study. Based on the observed activity of the test extracts of *Pleurotus ostreatus* in this study, the extracts which could be said to be bacteriostatic. The detected phytochemical constituents: carbohydrates, cardiac glycosides, and alkaloids in the aqueous ethanol extract may offer a rationale for the observed anti-mitotic and bacteriostatic activities as similar report on the immuno-modulatory ((Rop *et al* 2009) as well as anti-proliferative (Wandati *et al*, 2013) effects of the oyster mushroom polysaccharides beta glucan, and the antiviral activities of the oyster mushroom proteins (Jin-Yi, 2011; Piraino and Brandt 1999) are documented.

Table 2: Antibacterial susceptibility screening of the aqueous ethanol extract of *P. ostreatus*

	After 24 hours of incubation						After 48 hours of incubation				
Clinical strains of Pathogenic Organisms	AQE 1000 µg/mL	AQE 10 µg /mL	Growth control	Sterility control	Reference drug Levofloxacin 1µg/mL		AQE 1000 µg/mL	AQE 10µg /mL	Growth control	Sterility control	Reference drug Levofloxacin 1µg/mL
Escherichia coli	-	+	+	-	-		+	+	+	-	-
Pseudomonas aeruginosa	+	+	+	-	-		+	+	+	-	-
Klebsiella pneumonia	+	+	+	-	-		+	+	+	-	-
Staphylococcus aereus	+	+	+	-	-		+	+	+	-	-
Bacillus subtillis	-	+	+	-	-		+	+	+	-	-

KEY: AQE: Aqueous ethanol extract of *Pleurotus ostreatus;* + = Growth; - = No growth

CONCLUSION

This investigation revealed the anti-mitotic and bacteriostatic potential of *P. ostreatus* and thus validated its ethnomedicinal use in the treatment of tumours and related opportunistic bacterial infections. Further work is on-going to isolate and characterised the anti-mitotic constituents that could served as leads in the development of drugs and nutraceutical supplements for the treatment of cancer

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