IJPSR (2018), Volume 9, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 February, 2018; received in revised form, 11 July, 2018; accepted, 13 July, 2018; published 01 October, 2018

ASSESSING THE GENOTOXIC AND CYTOTOXIC RESPONSES OF THE H-29 CANCER CELL LINES ON THE ETHANOLIC EXTRACTS OF THE OYSTER MUSHROOM, *PLEUROTUS OSTREATUS* VAR. FLORIDA

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Keywords:

Genotoxicity, Cytotoxicity, HT-29 colon cancer cell lines, DNA damage, Apoptosis

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ABSTRACT: Globally, colorectal cancer is the third most common known today. Lack of cheap, rapid, safe and reliable cancer treatments resulted to the continued pursuit to find natural sources of bioactive compounds that can be cytotoxic or damaging to the DNA of cancer cells. One of the potential resource is the edible oyster mushroom (*Pleurotus ostreatus* var Florida) reported to have medicinal properties. Since many of the studies did not show specific varieties of the species used for the evaluation specifically on the anti-tumor and anticancer properties of the species, this study was therefore conducted. To determine whether there are cytotoxic and genotoxic effects of the ethanolic extracts of this variety of the white oyster mushroom against HT-29 colon cancer cell lines by damaging the DNA of cancer cells, the Comet assay method was used. This assay made use of differences in the measurements calculated by Open Comet Software of the tail length, tail DNA, tail DNA percent and tail moment in untreated and IC_{50} ethanolic extract-treated cells. Results of the statistical analysis of the profiles of cells show significant differences between the untreated and treated HT-29 cells. This simply implies that the ethanolic extracts from *P. ostreatus* var. Florida had induced DNA damage to HT-29 cancer cell lines and that this variety of the species of *P. ostreatus* is a potential source of bioactive compounds important for the treatment of colorectal cancer.

INTRODUCTION: Colorectal cancer is the third most common cancer globally predicted to increase in the coming years [1, 2]. In the Philippines; colon cancer surpassed liver cancer in recent statistics making it the leading type of cancer that Filipinos suffer from [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(10).4201-09

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(10).4201-09

The applications of chemotherapy and radiation methods are commonly advised but these are also toxic to normal cells leading not only to more human health complications but also to their cancer-inducing properties [4]. An efficient organic cancer drug therapy is being sought for thus encouraged researchers to continue to discover possible sources of natural products that are safer, cheaper and more reliable than synthetic drugs. Natural products from plants for example are gaining popularity as alternative ways to combat cancer. It is argued that many species provide reservoirs of natural chemicals without affecting normal cells and causing further complications [5].

Some of these are the edible mushrooms which are considered 'the ultimate health food' [6] not only because of their high nutritional value [7, 8, 9, 10] but also for their medicinal [11] and therapeutic applications [12]. Several studies have shown that these groups produce bioactive compounds [12] that are not only excellent antioxidants [13, 14, 15, 16] but also as having a wide range of therapeutic effects. Most compounds act as immune-modulatory [17, 18, 19], anticarcinogenic [20, 21, 22, 23], antibacterial and antiviral [24, 25, 26], anti-hypoglycaemic [27, 28, 29, 30, 31, 32], antiatherosclerotic [33] and anti-inflammatory agents [12, 34]. It is important to note however that different species of mushrooms vary in terms of the production of these compounds depending on the type of mushroom, substrate applied, cultivation and fruiting conditions, stage of development, age of the fresh mushroom, storage conditions, processing and cooking procedures [35].

One of the mushroom group of species that is very popularly used as food is the oyster mushroom (*Pleurotus spp.*) because of its taste, texture and unique aroma [36, 37]. This group is considered as good sources of dietary fiber and other valuable nutrients [38], antioxidant [39] and other medicinal properties. One of the species of this group *Pleurotus ostreatus*is reported to contain a number of biologically active compounds [40, 41]. Many of these compounds have therapeutic activities such as modulating the immune system [42, 43], inhibit tumor growth [44], anticancer [12, 45, 46, 47, 48] and anti-inflammation, have hypoglycemic and anti-thrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis [13, 49, 50, 51, 52, 53], and have anti-microbial and other activities [41, 54, 55, 56, 57].

These properties were extensive reviewed by Yashvant *et al*., [58]. What were observed from these studies however, were few studies involving the cytotoxicity and genotoxicity properties of specific varieties or subspecies of the different species of edible mushrooms such as *Pleurotus ostreatus* **Fig. 1**. This species has different varieties that are cultured and marketed. Since many studies that were conducted were not specifying what variety of the species were studied, this current study was conducted. The study focused on the Florida variety of *P. ostreatus* that is cultured and marketed in the Philippines.

The current study specifically assessed the genotoxic and cytotoxic properties of *P. ostreatus* var. Florida ethanolic extract to be able to understand the basis for many studies on the ethnomedicinal properties of this mushroom specifically on its anticancer properties. To be able to do this, we investigated the DNA damaging activity of the ethanolic extract of the mycelium of the mushroom against HT-29 colon cancer cell lines using comet assay and also evaluate its cytotoxicity using the Presto Blue Assay.

FIG. 1: WHITE OYSTER MUSHROOM *PLEUROTUS OSTREATUS* FLORIDA

METHODOLOGY: The protocol followed in this study is summarized in **Fig. 2**. The fungi samples of *Pleurotus ostreatus* were collected from a mushroom culture lab in Caloocan City, Philippines, and the fruit bodies were collected before or as the mushroom veils opened. After collection, the fungi samples were air-dried for 24 h, finely cut and 100 g was soaked in 1000 ml of 95% ethanol at room temperature for 48 h. This was then filtered and the ethanol removed through a rotary evaporator. The crude extract was further lyophilized to completely remove water from the sample.

The cellular responses of the HT-29 human colorectal adenocarcinoma cell lines obtained from Research and Biotechnology Division, St. Lukes Medical Center against the ethanolic extract was then evaluated. These cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco) added with 10% fetal bovine serum (FBS), Penicillin-Streptomycin 1% (v/v) placed in a tissue culture flask and incubated in 5% CO_2 at 37 °C. A cell count was done with a 1:4 dilution factor. 50 µl of the cell suspension was added with 150 µl trypan blue. Then, this was subjected to a hemocytometer and cell viability was determined.

The cells were then placed onto a 96-well plate and were incubated for 24 h in 5% CO_2 at 37 °C. The layout of the 96-well plate consisted of wells 2 from A to H having cells and the media (with Roswell Park Memorial Institute (RPMI) and 2% Fetal bovine serum (FBS). Wells 3-5 from A to H with cells diluted with the *Pleurotus ostreatus* extract in a 5-fold 10% DMSO dilution and the media. Wells 6-8 from A to H containing 100% DMSO and the cells diluted in 5-fold. For the negative control, wells 1 from A to H are placed with medium only. The 96-well plate was incubated for 24 h at 37 °C. In order to check the absorbance and IC_{50}, the concentration that kills 50% of the cancer cells, of the wells, a microplate reader (Synergy H4 Hybrid Reader) and a microplate software (Biotek Gen5 Data Analysis Software) were used. Prior to this, the incubated 96-well were washed and aspirated with new media, and placed with PrestoBlue for cell viability testing.

FIG. 2: A GRAPHICAL PRESENTATION OF THE STEPS TAKEN IN THE PREPARATION UP TO ANALYSIS OF THE EFFECTS OF THE ETHANOLIC EXTRACTS FROM *P. OSTREATUS* FLORIDA TO THE CANCER CELL LINES. a. Culture, b. Harvesting, c/ Drying, d. Cutting to small pieces, e. Soaking in ethanol, f. Rotvapping, g. Lyophilization, h. Cell culture, i. Cytotoxicity testing, j-l. Comet assay

The value of IC_{50} obtained from the cytotoxicity experiment was used as the concentration of the extract in the genotoxicity. Two sets of flasks of each cell line were subcultured where the first flask was treated with the extract and the other flask was left untreated. The flasks were incubated for 24 h at 36 ºC with 5% CO_2. After the incubation period, the cells were subjected to trypsinization to eradicate its adherence to the flasks. The cell suspensions were centrifuged at 1500 rpm for 7 min at room temperature. The supernatant was removed, and the cells (pellet) were washed with RPMI 1640 medium. Centrifugation of the cell suspension at 1500 rpm for 7 min at room temperature was done. The cells were then resuspended with ice-cold 1X PBS. The Comet assay was performed under semi-alkaline conditions based on the protocol of Comet Assay™ Kit from SBYR Green Trevigen Inc.

The working lysis solution was prepared and cooled in Coplin Jar at 4 ºC 20 min before the experiment proper. Low melting point agarose (LMA), with its cap loosened, was placed in a beaker with boiling water for 5 min. The bottle was then placed in a 37 ºC water bath for 20 min. This part of the experiment was crucial because the temperature of the agarose for the cells was controlled sensitively. The alkaline solution was done by combining 0.6 g of NaOH pellets, 250 µl of 200 mM EDTA, and 49.75 ml distilled H_2O. Using the tip of the pipette, 50 µl of the solution was immediately pipette onto the Comet Assay Slide ensuring the whole sample area was covered. The slides were placed in refrigerator for 10 min.

The slides were immersed in the prepared 4 ºC lysis solution for 60 min. Slides were then submerged in Alkaline Unwinding Solution at room temperature for 20 min. After lysis and unwinding, the comet slides were placed in an electrophoresis tank filled with TBE buffer. The electrophoresis was set to 21 V for 30 min. Subsequently, the slides were immersed twice in distilled water for 5 min.

The final rinse was with 70% ethanol for 5 min. The slides were stored in a storage unit. 100 µl of diluted SYBR® Green was placed onto each well of dried agarose. The slides were then incubated for 24 h. The slides were captured and viewed using fluorescence microscope with magnification of 10x. A minimum of four images were captured, with no overlapping capture areas. The tail length, tail DNA%, and tail moment were analyzed using the Open Comet plugin for the Image J software.

Usable cells were automatically selected from the images Average DNA damage results were calculated from 50 cells selected at random. Calculation for tail moment was used to measure the severity of damage by combining amount of DNA in the tail with the distance of migration. Mean values for each measurement generated standard error of the mean (SEM) and was calculated from the standard deviation. Data were graphically presented as box and whiskers plots, scatter plot and classification matrix to show differences between treatments.

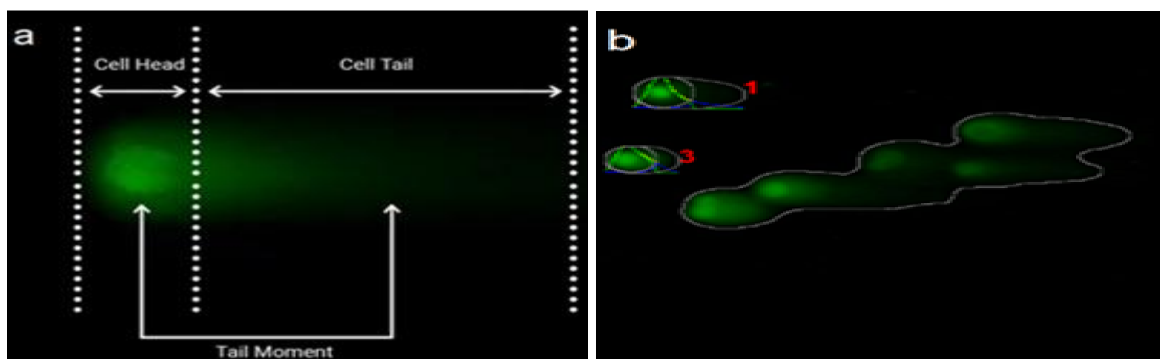

FIG. 3: COMET ANALYSIS SHOWING THE MEASUREMENT MADE ON TAIL LENGTH AND TAIL MOMENT (A) OVERLAPPING CELLS (B) WERE EXCLUDED FROM THE ANALYSIS

RESULTS AND DISCUSSION: The result of the determination of IC_{50} (mg/ml) of the *P. ostreatus* ethanolic extract obtained through PrestoBlue assay and used as basis for genotoxicity analysis of HT-29 colon cancer cell lines through Comet Assay is presented in **Fig. 4** and **Table 1**. The IC_{50} value was the inhibitory concentration at which 50 percent of the HT-29 colon cancer adenocarcinoma cell lines were terminated using the *P. ostreatus* extract. The IC_{50} value was used for the genotoxicity evaluation.

TABLE 1: IC_{50} OF THE ETHANOLIC EXTRACT BASED ON THE RESULT OF PRESTOBLUE ASSAY

Treatment	IC_{50} (mg/ml)	Absorbance
P. ostreatus ethanolic extract	164 mg/ml	610 nm

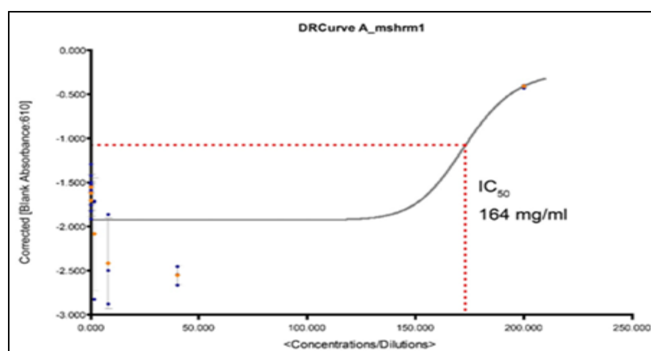

FIG. 4: THE GRAPH ABOVE SHOWS THE CORRECTED / NORMALIZED VALUES OF THE ABSORBANCE PER WELL. PLOTTED ON THE GRAPH ARE THE CORRECTED AVERAGE VALUES PER ROW OF EACH SET-UP OF CONCENTRATION

The Comet assay results to determine if the ethanolic extract could induce DNA damage and fragmentation of HT-29 colon cancer cell lines, the tail length, tail DNA, tail moment measured through the use of the Comet software are shown in **Table 2**, **Fig. 5** and **6**.

TABLE 2. COMET ASSAY SHOWING THE TAIL LENGTH, TAIL DNA, AVERAGE TAIL DNA PERCENT AND TAIL MOMENT BETWEEN TREATED AND UNTREATED HT-29 COLON CANCER ADENOCARCINOMA CELL LINES

Group	Average tail length	Average tail DNA	Average tail DNA%	Average tail moment
Culture media only (untreated)	16.40	25172.20	22.77	4.23
P. ostreatus ethanolic extract	51.38	39521.86	36.91	20.20

*the blue shaded area indicates the set-up for the fungi extract

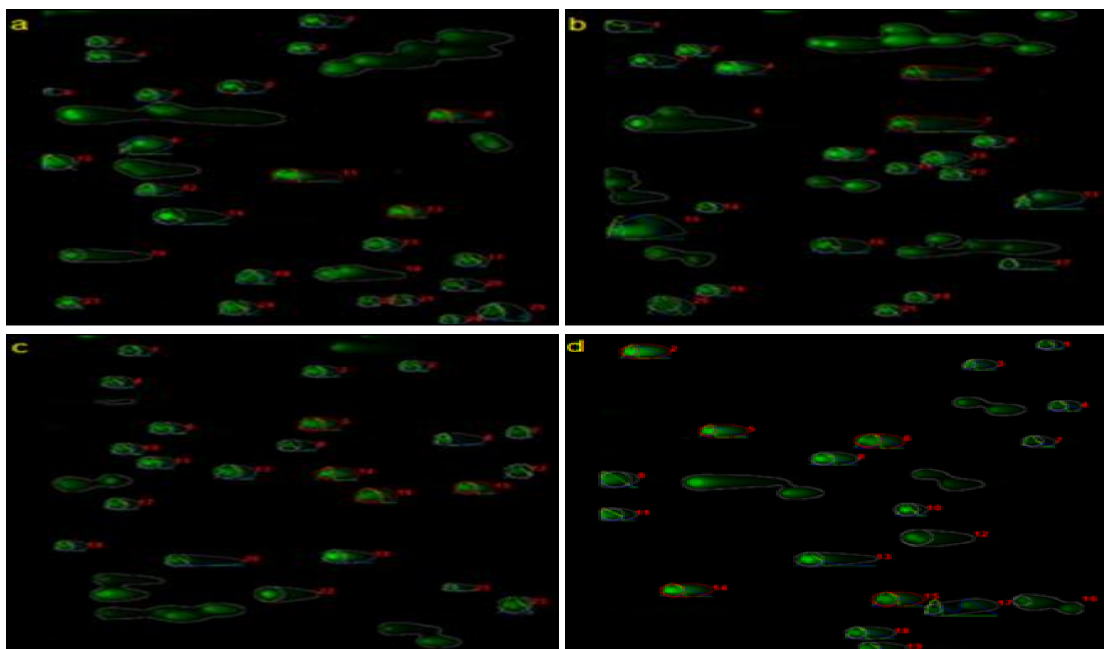

FIG. 5: IMAGES (a-d) OF TREATED HT-29 COLON CANCER ADENOCARCINOMA CELLS WITH *P. OSTREATUS* FLORIDA ETHANOLIC EXTRACTS (GREY OUTLINES SIGNIFY CELLS THAT HAVE BEEN DECIDED UNUSABLE BY THE SOFTWARE. THOSE WITH RED OUTLINES HAVE BEEN UTILIZED TO CALCULATE THE MEAN DATA. RED NUMBERS TAG THE CELL FOR IDENTIFICATION IN THE RAW DATA. THE ELONGATED CELLS SHOW THAT DAMAGE WAS DONE TO THE HT-29 CELLS BY THE EXTRACT)

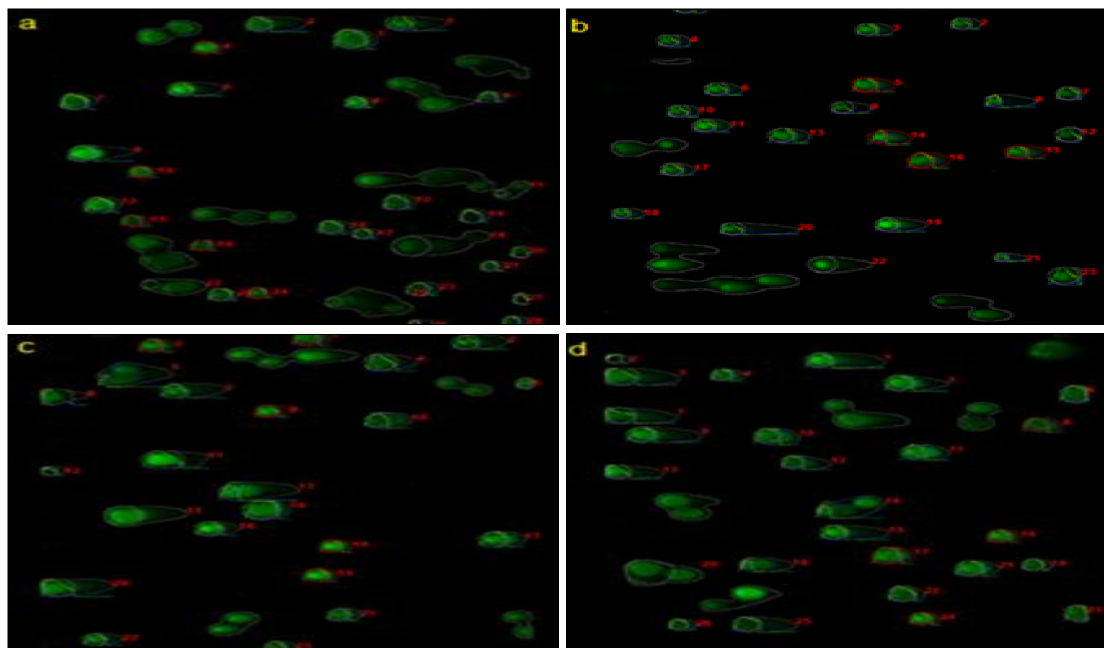

FIG. 6: IMAGES (a-d) OF UNTREATED HT-29 COLON CANCER ADENOCARCINOMA CELLS (GREY OUTLINES SIGNIFY CELLS THAT HAVE BEEN DECIDED UNUSABLE BY THE SOFTWARE. THOSE WITH RED OUTLINES HAVE BEEN UTILIZED TO CALCULATE THE MEAN DATA. RED NUMBERS TAG THE CELL FOR IDENTIFICATION IN THE RAW DATA. THE ELONGATED CELLS SHOW THAT DAMAGE WAS DONE TO THE HT-29 CELLS BY THE EXTRACT

Comparison between treated and untreated HT-29 colon cancer adenocarcinoma cell lines based on tail length, tail DNA, average tail DNA percent and tail moment subjected to CVA analysis show significant differences **Table 3**. The differences are graphically demonstrated in **Fig. 7** and **8**.

TABLE 3: COMPARISON BETWEEN TREATED AND UNTREATED HT-29 COLON CANCER CELL LINE (PILLAI TRACE: 0.5496, P=9.673E-16)

	Treated	Untreated
Treated	-	9.6731E-16
Untreated	9.6731E-16	-

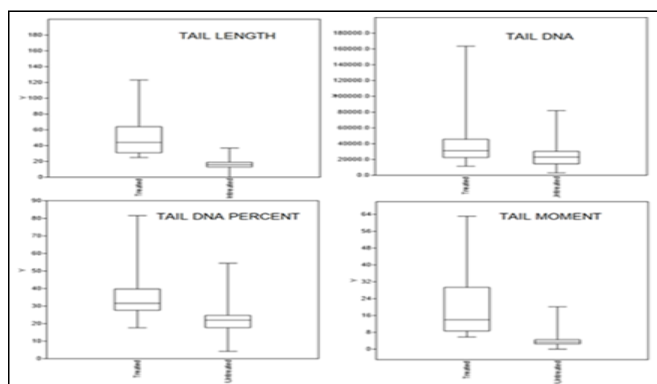

FIG. 7: BOX AND WHISKER PLOTS SHOWING THE MEAN AND STANDARD DEVIATIONS BETWEEN TREATED AND UNTREATED HT-29 COLON CANCER CELL LINE BASED ON TAIL LENGTH, TAIL DNA, TAIL DNA PERCENT AND TAIL MOMENT

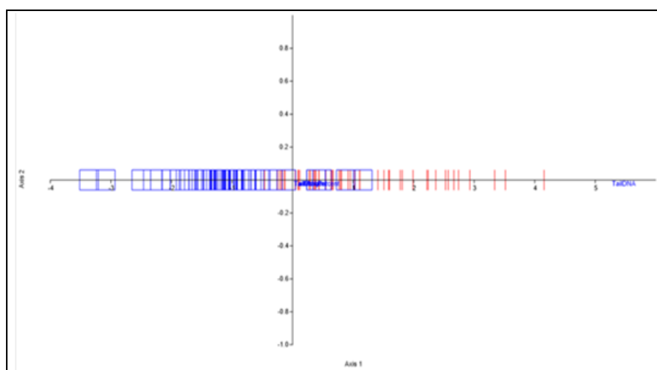

FIG. 8: SCATTERPLOT SHOWING THE DISTRIBUTION OF INDIVIDUALS TREATED AND UNTREATED HT-29 COLON CANCER CELL LINE

Comparing the treated and untreated HT-29 colon cancer adenocarcinoma cells show higher discrimination between the examined cell populations as shown in **Table 4**.

TABLE 4: CLASSIFICATION MATRIX BETWEEN TREATED AND UNTREATED HT-29 COLON CANCER CELL LINES

	Treated	Untreated	Total
Treated	43 (86%)	7 (14%)	50 (100%)
Untreated	5 (19%)	45 (90%)	50 (100%)

This current study showed similar results generated in a study conducted by Gu [59] on water-soluble extract from fresh *P. ostreatus* where the crude oyster extract has a cytotoxic effect on PC-3 cells and the aqueous polysaccharide extract has anti-proliferative and pro-apoptotic effects on HT-29 cells. Mohamed [45] and Mizuno [60] in their study showed the presence of bioactive compounds in *P. ostreatus* that are responsible not only for the reduction / prevention of hypertension, high cholesterol, anti-atherosclerosis, anti-viral and anti-thrombotic and immunomodulatory, but also anti-cancer [48, 61].The polysaccharides, terpenoids, fatty acids, amino acids, steroids, and phenols found in *P. ostreatus* are considered to be anticancer agents along with its glycoproteins such as lectins which are also responsible for anticancer and immune-stimulating activities.

Lavi and Friesem *et al.*, [62] show the anti-proliferative and pro-apoptotic activities of fractions from *P. ostreatus* against HT-29 colon cancer cells. Lavin *et al.*, [62] claimed from their study that *P. ostreatus* aqueous polysaccharide extract exhibited anti-proliferative and pro-apoptotic effect on HT-29 colon cancer cells. Jedinak and Sliva *et al.*, [20] also show in their study that methanolic extracts of *P. ostreatus* subdued the proliferation of breast cancer (MCF-7, MDA-MB-231) and colon cancer (HT-29, HCT-116) cells, without affecting the normal human epithelial mammary MCF-10A and normal colon FHC cells Jedinak and Sliva *et al.*, [20].

Ekowati *et al.*, [63] showed extracts from *P. ostreatus* inhibited the growth of *Helacyton gartleri* cells, making it cytotoxic to cervical cancer cells. The high cytotoxicity of the ethanolic extract against HT-29 colon cancer adenocarcinoma cell lines may also be attributed to the presence of selenium in *P. ostreatus.* Selenium in mushrooms is reported to be capable of reducing or affecting the metabolic activity of the cells by promoting DNA oxidation that leads to DNA strand breaks [64, 65] and in low concentrations possess anticarcinogenic properties while in higher concentrations may cause death of the cells [66]. These may also explain the results generated in the *P. ostreatus* var Florida.

While these published reports on this species of mushrooms dwell on the anticancer activities of

various extracts, it did not specify the exact variety of the species used. While it is argued that many or almost all organisms differ genetically especially on the variations of the production of metabolic compounds produced, the current study which explored if the Florida variety of *P. ostreatus* also show anticancer properties was no different from the abovementioned studies. This means that any variety of *P. ostreatus* will have similar anticancer bioactivity.

CONCLUSION: Results of the study show that the cytotoxic activity of the ethanolic extracts by the *P. ostreatus* var. Florida could explain the induced DNA damage to HT-29 colon cancer cell lines as shown by the differences of the presence of tail DNA, tail length and tail moment between the treated and untreated cells. The percent DNA damage obtained by the *P. ostreatus* var. Florida-treated cells indicate that the extract is highly genotoxic to HT-29 colon cancer cell lines. This study generally confirms previous investigations on *P. ostreatus* tested against other carcinoma cells. This study therefore clearly shows the potential of *P. ostreatus* Florida variety as source of bioactive compounds for use as anticancer agents.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Soerjomataram FJ, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: GLOBOCAN 2012 v1.1, Cancer Incidence and Mortality Worldwide: IARC Cancer Base no. 11 (Internet). Lyon, France: International Agency for Research on Cancer 2014. http://globocan.iarc.fr, accessed on 16/01/2015.
2. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ and Cronin KA: SEER Cancer Statistics Review, 1975-2014, National Cancer Institute. Bethesda, MD. http://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site.(April 2017).
3. Dy FT: Colorectal cancer now Philippines' number 1 cancer. 2017. http://www.philstar.com/health-and-family/2017/03/08/1679163/colorectal-cancer-now-philippines-number-1-cancer.
4. Ochwang'i DO, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM and Kiama SG: Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. J Ethnopharmacol 2004; 151(3): 1040-1055.
5. Taneja SC and Qazi GN: In: Bioactive molecues in medicinal plants: A perspective in their therapeutic action, in drug discovery and development. John Wiley and Sons, Inc, 2007: 1-50.
6. King TA: Mushrooms, the ultimate health food but little research in U. S to prove it. Mush News 1993; 41: 29-46.
7. Nylen B and Matsvampar V: Naturoch. Kultur/LTs forlag, 1985.
8. Manzi PL, Gambelli S, Marconi V, Vivandti V and Pizzoferrato F: Nutrients in edible mushrooms: An interspecies comparative study. Food Chem 1999; 65: 477-82.
9. Sanmee RB, Dell P, Lumyong K, Izumori K and Lumyong S: Nutritive value of popular wild edile mushrooms from northern Thailand. Food Chem 2003; 82: 527-32.
10. Hafiz F, Begum M, Parveen S, Nessa Z and AK Azad: Study of edible. Mushroom grown on *Eucalyptus camaldulensis* trunk and under soil of Albizzia procera. Pakistan J Nutr 2003; 2: 279-82.
11. Badalyan SM: Anti-tumor and immune-modulating activities of compounds from several basidiomycetes mushrooms. Probl Med Mycology 2000; 2: 23-28.
12. Wasser SP and Weis AL: Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives. Int J Med Mushr 1999; 1: 31-62
13. Wang Z, Luo D and Liang Z: Structure of polysaccharides from the fruiting body of *Hericium erinaceus* Pers. Carbohydrate Polym 2004; 57: 241-247. doi: 10.1016/j. carbpol.2004.04.018.
14. Costa OVJ, Garbi NMC, Asquieri RE: Nutritional value of *Agaricus sylvaticus*: mushroom grown in Brazil. Nutricion Hospitalaria 2012; 27: 449-455.
15. Cheung LM and Peter CK: Mushroom extracts with antioxidant activity against lipid peroxidation. Food Chemistry 2005; 89: 403-409.
16. Wasser SP and Weis AL: Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (Review). Int J Med Mushrooms 1999; 1: 47-50.
17. Mau JL, Chang CN, Huang SJ and Chen CC: Antioxidant properties of methanolics extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. Food Chemistry 2004; 87: 111-118.
18. Pan L, Pan H, Soccol AT, Pandey A and Soccol CR: Advances in mushroom research in the last decade. Food Technology and Biotechnology 2006; 44: 303-311.
19. Reguła J. and Siwulski M: Dried shitake (*Lentinulla edodes*) and oyster (*Pleurotus ostreatus*) mushrooms as a 19 good source of nutrient. Acta Science Polymer Technologie Alimentarius 2007; 6: 135-142.
20. Sliva D, Loganathan J, Jiang J, Jedinak A, Lamb JG, Terry C, Baldridge LA, Adamec J, Sandusky GE and Dudhgaonkar S: Mushroom *Ganoderma lucidum* prevents colitis-associated carcinogenesis in mice. PLoS ONE 7(10): e47873. https://doi.org/10.1371/journal.pone.0047873
21. Moradali MF, Mostafavi H, Ghods S and Hedjaroude GA: Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). Inter Immunopharmacol 2007; 7: 701-724.
22. Zong A, Cao H and Wang F: Anticancer polysaccharides from natural resources: a review of recent research. Carbohydr Polym 2012; 90(4): 1395-1410
23. Liu Y, Sun J, Luo Z, Rao S, Su Y, Xu R and Yang Y: Chemical composition of five wild edible mushrooms collected from Southwest China and their anti-hyperglycemic and antioxidant activity. Food Chem Toxicol 2012; 50: 1238-1244.

24. Janakat S, Al-Fakhiri S and Sallal AK: A promising peptide antibiotic from *Terfezia claveryi* aqueous extract against *Staphylococcus aureus in-vitro*. Phytotherapy Research 2004; 18, (10): 810-813.
25. Janakat S, Al-Fakhiri S and Sallal AK: Evaluation of antibacterial activity of aqueous and methanolic extracts of the truffle *Terfezia claveryi* against *Pseudomonas aeruginosa*. Saudi Med J 2005; 26(6): 952-5.
26. Adotey G, Quarcoo A, Holliday JC, Fofie S and Saaka B: Effect of immunomodulating and antiviral agent of medicinal mushrooms (Immune Assist 24/7TM) on CD4+ T-lymphocyte counts of HIV infected patients. Int J Med Mushr 2011; 13: 109-113.
27. Hu SH, Wang JC, Lien JL, Liaw ET and Lee MY: Antihyperglycemic effect of polysaccharide from fermented broth of *Pleurotus citrinopileatus*. Appl Microbiol Biotechnol 2006a; 70: 107-113
28. Hu SH, Liang ZC, Chia YC, Lien JL, Chen KS, Lee MY and Wang JC: Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. J Agric Food Chem 2006b; 54: 2103-2110.
29. Hu T, Liu P, Ni Y and Lu C: Isolation, purification and effects of hypoglycemic functional polysaccharides from *Inonotus obliquus*. African J Biotechnol 2012; 11: 7738-7743.
30. Kwon AH, Qiu Z, Hashimoto M, Yamamoto K and Kimura T: Effects of medicinal mushroom (*Sparassis crispa*) on wound healing in streptozotocin-induced diabetic rats. Am J Surg 2008; 197 (4): 503-509.
31. Kwon JS, Lee JS, Shin WC, Lee KE and Hong EK: Optimization of culture conditions and medium components for the production of mycelial biomass and exo-polysaccharides with *Cordyceps militaris* in liquid culture. Biotechnol Bioprocess Eng 2009; 14: 756-762.
32. Lo HC and Wasser SP: Medicinal mushrooms for glycemic control in diabetes mellitus: history, current status, future perspectives, and unsolved problems (review). Int J Med Mushr 2011; 13: 401-426.
33. Guillamón E, García-Lafuente A, Lozano M, D'Arrigo M, Rostagno MA, Villares A and Martínez JA: Edible mushrooms: role in the prevention of cardiovascular diseases. Fitoterapia 2010; 81: 715-723.
34. Chang S and Miles GP: Mushrooms cultivation, nutritional value, medicinal effects and environmental impact. Boca Raton, FL CRC Press 2004: 436.
35. Rajewska J and Balasinska B: Biologically active compounds of edible mushrooms and their beneficial impact on health. Postepy Higieny Medycyny Doswia-dczaine 2004; 58: 352-357.
36. Chang ST and Buswell JA: Mushroom nutriceuticals. World Journal of Micorbiology and Biotechnology 1996; 12(5): 473-476.
37. Arbaayah HH and Umi KalsomY: Antioxidant properties in the oyster mushrooms (*Pleurotus* spp.) and split gill mushroom (*Schizophyllum commune*) ethanolic extracts. Mycosphere, Journal of Fungal Biology 2013: 4(4): 661-673.
38. Cohen R, Persky L and Hadar Y: Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. Appl Microbiol Biotechnol 2002; 58: 582-594.
39. Jayakumar T, Thomas PA, Sheu JR and Geraldine P: *In-vitro* and *in-vivo* antioxidant effects of the oyster mushroom *Pleurotus ostreatus*. Food Res Int 2011; 44: 851-861.
40. Patel Y, Naraian R and Singh VK: Medicinal Properties of *Pleurotus* Species (Oyster Mushroom): A Review World Journal of Fungal and Plant Biology 2012; 3(1): 01-12.
41. Oyetayo VO and Ariyo OO: Antimicrobial and antioxidant properties of *Pleurotus ostreatus* (Jacq: Fries) cultivated on different tropical woody substrates. J Waste Conv Bioprod Biotechnol 2013; 1: 28-32. doi: 10.5147/jwcbb. 2013.0121
42. Jeurink PV, Cristina LN, Huub FJS and Harry JW: Immunomodulatory capacity of fungal proteins on the cytokine production of human peripheral blood mono-nuclear cells. Intl. Immunopharmacol 2008; 8: 1124-1133.
43. Yilmas N, Solmaz M, Turkekul I and Elmastas M: Fatty acid composition in some wild edible mushrooms growing in the middle Black Sea region of Turkey. Food Chem 2006; 99: 168-174.
44. Nanba H: Maitake mushroom-the king mushroom. Mush News 1993; 41(2): 22-25.
45. Mizuno T: The Extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan (Review). Int J Med Mushrooms 1999; 1: 9-29.
46. Wang D, Sakoda A and Suzuki M: Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. Bioresour Technol 2001; 78: 293-300.
47. Wang H, Gao J and Ng TB: A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*. Biochem Biophys Res Commun 2000; 275: 810-6.
48. Mostafa M: Chemical profile, agaritine content and selenium in *Agaricus bisporus*. Braz Arch Biotechnol 2012; 55: 911-20.
49. Barros L, Baptista P, Estevinho LM and Ferreira ICFR: Bioactive properties of the medicinal mushroom *Leucopaxillus giganteus* mycelium obtained in the presence of different nitrogen sources. Food Chem 2017; 105: 179-186.
50. Kim HG, Yoon DH, Lee WH, Han SK, Shrestha B, Kim CH, Lim MH, Chang W, Lim S, Choi S, Song WO, Sung JM, Hwang KC and Kim TW: *Phellinus linteus* inhibits inflammatory mediators by suppressing redox-based NF-kappa B and MAPKs activation in lipopolysaccharide-induced RAW 264.7 macrophage. J Ethnopharmacol 2007; 114(3): 307-15.
51. Tong H, Fengguo X, Kai F, Guangren S, Xiaoxv G, Liwei S, Rui J, Dan T and Xin S: Structural characterization and *in-vitro* antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus* Bioresource Technology 2009; 100: 1682-1686.
52. Synytsya A, Mickova K, Synytsya A, Jablonsky I, Spevacek J and Erban V: Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: structure and potential prebiotic activity. Carbohydr Polym 2009; 76: 548-556. doi: 10.1016/j. carbpol.11.021.
53. Fontes Vieira PA, Gontijo DC, Vieira BC, Fontes EAF, Soares de Assunção L, Leite JPV, Goreti de A. Oliveira M and Kasuya MC: Antioxidant activities, total phenolics and metal contents in *Pleurotus ostreatus* mushrooms enriched with iron, zinc or lithium-LWT. Food Science and Technology 2013; 54: 421e425.
54. Gunde-Cimerman N and Cimerman A: *Pleurotus* fruiting bodies contain the inhibitor of 3-hydroxy-3-methylglutaryl -coenzyme a reductase-lovastatin. Exp Mycol 1995; 19: 1-6.
55. Badalyan SM: The main groups of therapeutic compounds of medicinal mushrooms. Probl Med Mycology 2001; 3: 16-23.

56. Lindequist U: The pharmacological potential of mushrooms. Ev Based Comp Alt Med 2005; 2: 285-299.
57. Wasser SP: Medicinal mushroom science: history, current status, future trends, and unsolved problems. Int J Med Mushr 2010; 12: 1-16.
58. Yashvant P, Ram N and Singh VK: Medicinal properties of *Pleurotus sp.* (Oyster mushroom): A review. World Journal of Fungal and Plant Biology 2012; 3(1): 01-12.
59. Gu, YH and Sivam G: Cytotoxic effect of oyster mushroom *Pleurotus ostreatus* on human androgen-independent prostate cancer PC-3 cells. J Med Food 2006; 9: 196-204.
60. Mohamed M and Eman FA: Bioactive compounds of fresh and dried *Pleurotus ostreatus* mushroom. Lifescience Global 2014; 3(1): 4-14.
61. Solmaz G, Ozen F, Ekinci Y, Bird PS and Korachi M: Inhibitory and disruptive effects of shiitake mushroom (*Lentinula edodes*) essential oil extract on oral biofilms. Jundishapur J Microbiol 2013; 6(9): e9058.
62. Lavi I, Friesem D, Geresh S, Hadar Y and Schwariz B: An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. Cancer Letters 2006; 244: 61-70.
63. Ekowati Y, van Diepeningen A, Ferrero G, Kennedy MD, Husman AMR and Schets FM: Clinically relevant fungi in water and on su rfaces in an indoor swimming pool facility. International Jour of Hygiene and Environmental Health 2017; 220 (7): 1152-1160.
64. Lu J, Kaeck M, Jiang,C, Wilson A and Thompson HJ: Selenite induction of DNA strand breaks and apoptosis in mouse leukemic L1210 cells. Biochem Pharmacol 1994; 47: 1531-1535.
65. Zhou N, Xiao H, Li TK, Kamal E and Liu L: DNA damage-mediated apoptosis induced by selenium compounds. J. Biol. Chem. 2003; 278: 29532-29537.
66. Spallholz JE: On the nature of selenium toxicity and carcinostatic activity. Free Radic Biol Med 1994; 17: 45-64.

How to cite this article:

Buniel PAS, Scheewe HWP, Jr CGS, Alima ZD and Demayo CG: Assessing the genotoxic and cytotoxic responses of the H-29 cancer cell lines on the ethanolic extracts of the oyster mushroom, *Pleurotus ostreatus* var. Florida. Int J Pharm Sci & Res 2018; 9(10): 4201-09. doi: 10.13040/IJPSR.0975-8232.9(10).4201-09.

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