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Test the Cytotoxicity of Pleurotin Extracted from an Edible Mushroom *Pleurotus osteratus* Against Three Human Carcinoma Cell Line

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Abstract

The study included selection six species of the fungi related to *Pleurotus* genus were evaluated for their ability to production of Pleurotin, one of them, *Pleurotus ostreatus* (P.11) was isolated and identified in the present study. Pleurotin was extracted with screening by Thin Layer Chromatography (TLC) and quantification High Performance Liquid Chromatography (HPLC). Cytotoxicity of Pleurotin extracted from *P. ostreatus* (P.11) grown in different sugar sources (galactose, mannitol, sucrose, dextrose and lactose) liquid media was test against three selected cancer cell lines, CaSki, MCF-7 and A549 addition to Human Non Cancer Fibroblast Cell Line (MRC-5). Pleurotin of *P. ostreatus* (P.11) grown in galactose induced the significant highest growth inhibition against all three cancer cell lines MCF-7 CaSki, and A549 at 72 h treatment period with IC_{50} 29.84 ± 2.37 , 30.25 ± 2.40 and 37.60 ± 2.65 $\mu\text{g/ml}$ respectively when the $P \leq 0.01$, while it showed no adverse effect on the non-cancer human fibroblast cell line MRC-5 with $IC_{50} > 200$ $\mu\text{g/ml}$. Cytotoxicity of Pleurotin was compared with cytotoxicity of the positive controls (chemotherapeutic drugs) including Doxorubicin against CaSki and A549 cell lines and Cisplatin against MCF-7 and MRC-5 cell lines, although IC_{50} of pleurotin was higher (30.25 ± 2.40 and 37.60 ± 2.65 $\mu\text{g/ml}$) than Doxorubicin (0.18 ± 0.00 and 1.10 ± 0.02 $\mu\text{g/ml}$) of CaSki and A549 cell lines, respectively, and also IC_{50} of Pleurotin was higher (29.84 ± 1.73 and >200 $\mu\text{g/ml}$) than Cisplatin (8.20 ± 0.25 and 8.88 ± 0.13 $\mu\text{g/ml}$) of MCF-7 and MRC-5 cell lines, respectively, pleurotin was natural product from an edible fungus while Doxorubicin and Cisplatin were chemical drugs.

Keywords: Pleurotin, cytotoxicity, human breast carcinoma cell line (MCF-7), *Pleurotus ostreatus*.

اختبار الفعالية السمية للبليروتين المستخلص من الفطر الغذائي *Pleurotus osteratus* ضد ثلاث خطوط من الخلايا السرطانية

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الخلاصة

شملت الدراسة انتخاب ستة انواع من الفطريات العائدة لجنس *Pleurotus* وتقييم قدرتها على انتاج البليروتين وكان أحد هذه الانواع (*P. ostreatus* (P.11) والذي تم عزله وتشخيصه في الدراسة الحالية. و تم استخلاصه والتحري عن وجوده بواسطة صفائح الكروموتوغرافيا الرقيقة TLC وتقدير كميته بواسطة جهاز الكروموتوغرافي السائل ذو الاداء العالي HPLC. كما تم اختبار الفعالية السمية للبليروتين المنتج من الفطر *P. ostreatus* (P.11) المنمى على اوساط تحتوي على مصادر مختلفة للسكريات (كالكتوز، مانيتول، سكروز، دكستروز و اللاكتوز) على ثلاثة انواع من خطوط الخلايا السرطانية MCF-7، CaSki و A549 فضلاً عن خط خلايا الرئة الاعتيادية غير السرطانية (MRC-5). البليروتين المنتج من الفطر *P. ostreatus* (P.11) المنمى على وسط يحتوي كالكتوز اعطى اعلى تثبيط معنوي لنمو جميع خطوط الخلايا السرطانية الثلاثة MCF-7 و CaSki و A549، وفي مدة حضانة 72 ساعة ومع قيمة $IC_{50} = 2.37 \pm 29.84$ و 2.40 ± 30.25 و 2.65 ± 37.60 ميكروغرام /مل على التوالي عندما $P \geq 0.01$ ، بينما لم يظهر اي تأثير وخيم على خلايا الرئة الاعتيادية MRC-5 وقيمة لل $IC_{50} < 200$ ميكروغرام /مل. حيث تم اختبار الفعالية السمية للبليروتين مع السيطرة الموجبة (ادوية العلاج الكيميائي) المتضمنة الدوكسوروبيسين (ضد خط خلايا عنق الرحم السرطانية وخط خلايا الرئة السرطانية) والسيسيلاتين (ضد خط خلايا الثدي السرطانية وخط خلايا الرئة الاعتيادية)، وعلى الرغم من IC_{50} للبليروتين (2.40 ± 30.25 و 2.65 ± 37.60 ميكروغرام /مل) اعلى من الدوكسوروبيسين (0.00 ± 0.18 و 0.02 ± 1.10 ميكروغرام/مل) على خط خلايا عنق الرحم السرطانية وخط خلايا الرئة السرطانية على التوالي، وكذلك اعلى (1.73 ± 29.84 و 0.00 ± 200 ميكروغرام /مل) من السيسيلاتين (0.25 ± 8.20 و 8.88 ± 0.13 ميكروغرام /مل) على خط خلايا الثدي السرطانية و خط خلايا الرئة الاعتيادية على التوالي، الا ان البليروتين كان منتجاً طبيعياً من فطر صالح للاكل بينما الدوكسوروبيسين و السيسيلاتين من العقاقير الكيميائية.

Introduction

Cancer is a major public health problem worldwide [1]. It is amongst the three most common causes of death and morbidity in the world [2]. Some report from different cities of Iraq had showed increased incidence of different types of cancer, [3] showed the number of cancer cases registered was 5720 cancer in Iraq (31.05) case per 100,000 in 1991 to 14,180 case (44.46) per 100,000 population in 2008. These have been blamed on mutagenic and carcinogenic agents (like depleted uranium) employed in the wars of 1991 and 2003 [4]. *Pleurotus* mushrooms, they are healthy foods, low in calories and in fat, rich in protein, chitin, vitamins and minerals [5]. Important characteristics are observed concerning other chemical compounds, for example polysaccharides, mannans and β -glucans [6] and Pleurotin: is a naphthoquinone antibiotic, is a fungal metabolite found in extracts of some mushrooms such as *Pleurotus griseus*, as well as *Hohenbruehelia geogenius* and *Hohenbruehelia atrocaerulea*. Pleurotin as an anticancer lead compound was stimulated by the discovery that it inhibits the thioredoxin–thioreductase system. A potent inhibitor of the thioredoxin-thioredoxin reductase system (IC_{50} 170 nM). In the National Cancer Institute (NCI) anticancer 60 cell line testing panel pleurotin has an LC50 of 42 mM without selectivity toward a tissue type, thus failing to meet selection criteria [7]. The aims of this study was: Preliminary screening of Pleurotin against selected human carcinoma cell lines (Ca Ski, A549 and MCF-7) and a non-cancer fibroblast cell line (MRC5) using SRB assay.

Materials and Methods

Source of *Pleurotus* spp.

Six species of the fungi were obtained from the Mushrooms Production Unit in the College of Agriculture, University of Tikrit. The original sources of these fungi were *Pleurotus eryngii* (King oster-KING) cod: P.w.3, *Pleurotus djamar* (Pink oster-PK) cod:P. ix, *Pleurotus ostreatus* (Bluel grey-BG) cod: P. o. G, *Pleurotus ostreatus* (White oyster-White) cod: P. white, (all four species from Mushroom Box, U.K.) *Pleurotus ostreatus*, cod: P.o. 2 (from India type culture collection/India), *Pleurotus ostreatus* (locally) cod: P. 11(Locally (isolated in the present study/Iraq).

All fungal strains were routinely maintained on (PDA) slants, then kept refrigerated at 5°C and sub culturing at least monthly.

Laboratory Prepared Media

Modified Czapek's Dox liquid medium

Medium prepared by dissolving 40 g dextrose with 2 g sodium nitrate, 1g dipotassium phosphate, 0.5 g magnesium sulfate, 0.5 g potassium chloride, 0.01g ferrous sulfate and 5 g corn powder in one liter of D.W. and sterilized for 15 mins [8].

The source of sugar in this media was replaced with 4 types of sugar, each sugar separately (Galactose, Mannitol, Sucrose and Lactose) and tested on the cell cancer lines.

Cultivation of *P. ostreatus* in Liquid media

Czapek-Dox liquid media were added in clean Erlenmeyer flasks (500-mL capacity) each flask contain 150-200 ml media, then cover by cotton plug and sterilization in autoclave for 15 min. after cooling, the media were inoculated by piece (1×2 cm) of new growth colony (7 days) of *Pleurotus* spp., then incubated at 25-27 °C until the growth must be fully cover media surface.

Purification of Pleurotin

A. Extraction method by Chloroform

According to [8] the Pleurotin was purified as below, liquid media was filtered using gauze and cotton to remove the fungal mycelium. Then the filtrate was extracted with chloroform (1:1 v/v). The two layers were separated in separating funnel. The chloroform layer was extract by 1% sodium bicarbonate three times to acids remove. The extracting solvent was transfer to small baker and left at room temperature to evaporate all chloroform. Gummy redness substance was form this substance was dissolve by added 2-5 ml hot absolute ethanol. The baker was cover and left in room temperature to evaporate solvents. The formed crystals were washing by 2-5 ml ether and dried by air. The products was recrystallization by added 1:1 chloroform, ether then left to formed yellowish amber crystals this is pure pleurotin.

B. Extraction method by ethyl acetate (EtOAc)

Liquid media was filtered using cotton to remove the fungal mycelium. Then the filtrate was extracted with ethyl acetate (EtOAc) in 1:1 ratio of filtrate to ethyl acetate. Two layers were separated in separating funnel. The ethyl acetate layer was dried with anhydrous sodium sulphate and then filtered with Whatman filter paper. The extracting solvent was evaporated using rotary evaporator. The extract was then transferred in to a pre-weighed scintillation vial with small amount of chloroform. The vial was left in the fume hood to allow the removal of chloroform via evaporation. The vial was weighed after the chloroform was completely evaporated to determine the weight of the extract obtained [7].

Detection of purified Pleurotin

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was performed to determine a suitable solvent system for the isolation of chemical compound (pleurotin) from the bioactive fractions of *Pleurotus* spp. Precoated silica gel 60F254 TLC plates were utilized for TLC analysis. A small drop of sample and standard were spotted about 1.0 cm from the bottom of the TLC plate by using a capillary tube. When the sample had dried, the TLC plate was placed into a TLC developing tank filled with premixed organic solvent (3ml acetone + 7ml Hexan) system. The TLC plate was removed from the developing tank when it was fully developed to solvent front. Then it was dried by using hair blower and then examined in the UV-cabinet under length wave 430 nm Visible light. The TLC plate was then placed into an iodine vapor chamber to stain the separated chemical compounds' spots [9].

High Performance Liquid Chromatography (HPLC)

Column: 3µm particle (50 × 4.6 mm I.D) C-18 column. Mobile phase: solvent A: 0.1% ammonium format in ethanol, solvent ethanol, near gradients 0% B to – 100% B at 5 min. UV set at 210 nm. Flow rat 1.4 ml/min., temp. 35 °C. HPLC of the samples was performed with the HPLC system equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable UV-VIS detector (Shimadzu SPD-10 AVP) and C-R6A chromatopack data processors. Samples were centrifuged (2000 rpm for 2 min).

Then filtered through a single-use 0.22 µm nylon syringe filter (Aldrich) pass through disposable filter 2.5 µm prior to analysis on HPLC system under optimum separation condition, the concentration of sample were quantitatively determined by comparison the peak area of authentic standard with the

peak area of samples under the same separation condition. The sequences of the eluted material of the standard wear as follow, each standard was 10 µg/ml. Retention time of pleurotin 2.45 minute with 9126 area.

Calculation:-The concentration of the sample measuring according to the following equation:

$$\text{Concentration of sample } \mu\text{g/ml} = \frac{\text{area of sample}}{\text{area of standard}} \times \text{Conc. of standard} \times \text{Dilution Factor.}$$

Cytotoxicity Assay

All the cytotoxicity experiments rounded in HIR Laboratory for Functional Molecules, institute of Biological Science, University of Malaya, Malaysia, from 1 April 2014 to 1 September 2014.

Cell Lines in Cell Culture

The study was evaluated against three human cancer cell lines and one human normal cell lines as described below, which were obtained from the American Type Culture Collection (ATCC, USA) according to previously described protocols slight modifications [10].

- 1) Human Cervix Epidermoid Carcinoma Cell Line (CaSki)
- 2) Human Lung Carcinoma Cell Line (A549)
- 3) Hormon-Dependent Breast Adenocarcinoma Cell Line (MCF-7)
- 4) Human Non Cancer Fibroblast Cell Line (MRC-5)

The viability of the cells was checked before and after treatment by the trypan blue exclusion dye method. Frozen cell stocks were stored in vapor liquid nitrogen (-120 °C) prior to use.

Maintenance of Cells

The Human Cervix Epidermoid Carcinoma Cell Line (CaSki), Human Lung Carcinoma Cell Line (A549) and Hormone-Dependent Breast Adenocarcinoma Cell Lines (MCF-7) were maintained in RPMI 1640 Medium; Human Non-cancer Fibroblast Cell Line (MRC-5) in EMEM medium. The cell lines were incubated in 25 ml tissue culture flask at 5% CO₂ incubator kept at 37°C with humidified atmosphere. The 10% culture medium was replenished 5-10 ml every 2 or 3 times per week and routinely observed under an inverted microscope for any contamination [9 and 10].

Sulforhodamine B (SRB) Assay

The cytotoxicity activities of the pleurotin extracted from media containing different types of sugars were screened by using the sulforhodamine B (SRB) assay [11]. The CaSki, MCF-7, A549 and MRC-5 cells were cultured in the tissue culture flasks until it reached 60-70% confluence. The confluence of the tissues could be observed under the inverted light microscope. Removed the culture medium and washed the flask with PBS twice. Then, the cells were detached from the flask with 0.25% solution of Accutase enzyme and left the flask for 10 minutes. The cell pellet was obtained at 1000 rpm for 5 minutes. The density of the viable cells was counted by 0.4 % trypan blue exclusion in a hemocytometer.

Cells were then plated in 96-well microliter plate and incubated in a CO₂ incubator at 37°C for 24 hours to allow the cells to adhere and achieve 60-70% confluence at time of the time of the addition of the pure compounds. After 24 hours, the pure compound was then added to the well at six different concentrations of 3.13, 6.25, 12.5, 25, 50, 100 and 200µg/ml of pure compounds. And negative control is the well with the untreated cells. Then, the plates were incubated with the cells for 24, 48 and 72 hours for three different plates.

After the incubation, 50µl of 40% cold Trichloroacetic acid (TCA) was added to each well without removed the cell culture supernatant and incubates the plate at 4°C for 1 hour. After an hour, washed the plate five times with distilled water and the excess the water was removed by using the paper towels. Then, Sulforhodamine B (SRB) Dye was added to each well and left at room temperature for 30 minutes and then quickly rinsed the plate five times with 1% acetic acid to removed unbound dye. The excess acetic acid was removed by using paper towels. 100 µl of 10M Tris base solution to each well and shook the plate on a gyratory shaker for 5 minutes to solubilize the protein-bound dye. Then, the dye absorbance was measured at 556 nm microplate reader. Although the maximum absorbance of SRB can be achieved at 564nm, it is recommended that suboptimal wavelength of 490-530 nm should be used to avoid measurement in a nonlinear portion of the range. Alternatively, the amount of SRB can measured fluorometrically at excitation and emission wavelengths of 492 and 620 nm respectively and calculate the different between the two absorbance values.

The percentage of inhibition of each of the pure compounds was calculated according to the following formula:

$$\text{Percentage of inhibition} = \frac{\text{OD control} - \text{OD pure compounds}}{\text{OD control}}$$

Not: OD = Optical Density

Where,

OD_{pure compounds} = absorbance of the sample as read by the spectrophotometer at 556 nm.

OD_{control} = absorbance of untreated cells at 556 nm.

Cytotoxicity activity of pleurotin is expressed as IC₅₀ values (µg/ml). The IC₅₀ values are the concentration of test compounds that cause 50% inhibition of cell population or cell death, which was obtained by plotting the graph percentage of inhibition versus concentration of test sample or test compounds [12, 2 and 10].

Statistical Analysis

The Statistical Analysis System-SAS [13] was used to effect of different factors on study parameters. Least Significant Difference-LSD test was used to significant compare between means and Chi-square test was used to significant compare between percentages in this study.

Result and Discussion

Cultivation of *P. ostreatus* in Liquid media

After the inoculation of the modified Czapek-Dox liquid media with deferent types of natural additions by *Pleurotus ostreatus* for 4 to 8 weeks the growth of *Pleurotus* was fully covered media surface by white mycelium figure-1 if the development is ideal circumstances. This result is consistent with study of [14] stated that *P. ostreatus* ARC280 was grown on liquid media under static culture condition for thirty-one days of incubation.



Figure 1- Growth of *P. ostreatus* mycelium on modified Czapek-Dox liquid medium after 8 weeks.

Purification of Pleurotin by chloroform and ethyl acetate

When the purification process of pleurotin by chloroform and ethyl acetate done, pleurotin (Amber / yellow colored) crystals were formed after evaporation of the solvent, determinate by sensitive balance, their weight equal about 110 to 150 mg for each liter. This is agreeing with [8] in his study (Antibiotic substances from Basidiomycetes I. *Pleurotus griseus*) found pleurotin fine needle-like yellow crystals were formed as a result of rapid evaporation of the solvents. From 100 to 180 mg. of the crystalline material were obtained per l. of culture fluid. This result disagrees with [7] study when stated that pleurotin typically reaching ~300 mg/l at 5 weeks was shaken in the dark at 215 rpm at room temperature.

Detection of Purified Pleurotin

Thin Layer Chromatography (TLC)

In this study Thin Layer Chromatography (TLC) was used to determine a suitable solvent system for detection of pleurotin from the bioactive fraction of *P. ostreatus*.

High Performance Liquid Chromatography (HPLC)

After two months growth of *P. osteratus*, a chloroform extract was made from each whole ferment and examined by reverse phase HPLC with diode array detection and comparison of retention time with an authentic standard for the presence of pleurotin. Pleurotin concentration was measured in chloroform extract was found equal to 6 µg/ml (ppm) compared with standard which equal 10 µg/ml (ppm) Figure-2 showed presence pleurotin peak in chloroform extract from modified Czapek-Dox liquid media after completion growth of *P. osteratus*.

Ideally any extract should be defined so that any unknown extracts can be compared to check that the composition is the same. TLC or HPLC profiles best define extracts chromatographically [2].

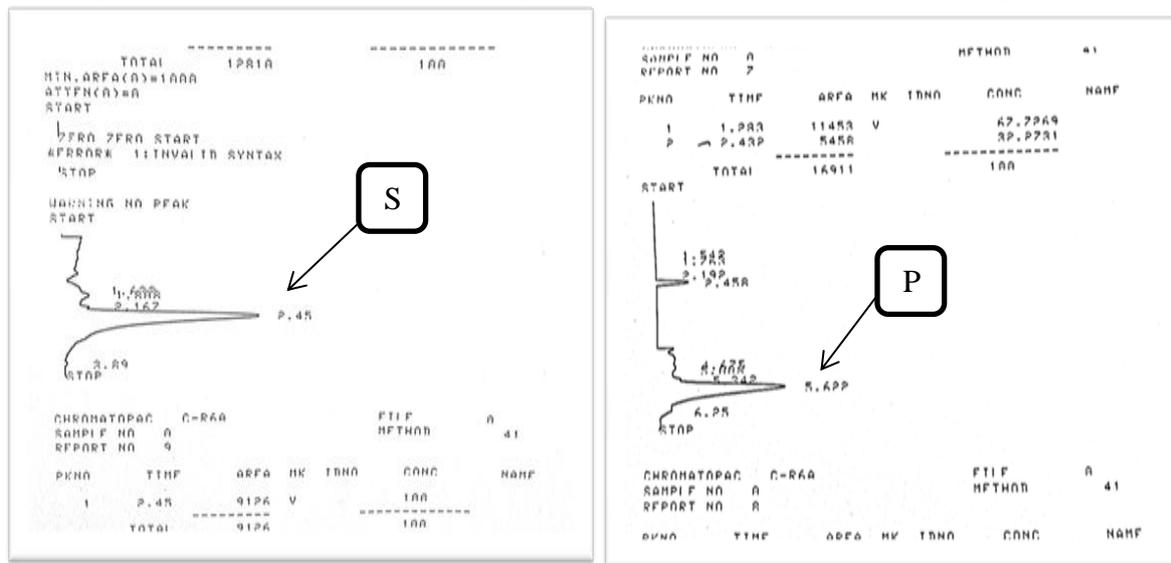


Figure 2- HPLC analysis of pleurotin standard (S) and chloroform extract of *P. ostreatus* shows the pleurotin peak in this extract (P).

Cytotoxicity of Pleurotin against Human Breast Carcinoma Cell line (MCF-7)

Cytotoxicity of pleurotin from *P. ostreatus* (P.11) grown in different sugars sources liquid media were tested against human breast carcinoma cell line (MCF-7). Pleurotin of *P. ostreatus* grown in galactose and mannitol induced the significant highest growth inhibition against MCF-7 cells when incubated 72h ($P \leq 0.01$). Following galactose and mannitol, sucrose, lactose and dextrose induced lower growth inhibition rate against the cancer cells Figure-3. Table-1 summarized the IC_{50} values of pleurotin extracted from liquid media contained different sugar sources. Galactose and mannitol had the lower IC_{50} values with 29.84 ± 2.37 and 48.90 ± 3.74 µg/ml respectively, followed by sucrose 65.2 ± 3.26 µg/ml, lactose 128.8 ± 9.42 µg/ml and dextrose $>200 \pm 0.00$ µg/ml. Results showed significant inhibition rate percentage was working in a dose-dependent manner ($P \leq 0.01$). [15] Proved in his study pleurotin have ability to decrease HIF-1 α protein levels and the expression of downstream target genes. pleurotin also decreased HIF-1 α protein levels and HIF-1 β transactivation in RCC4 renal cell carcinoma cells that constitutively overexpress HIF-1 α protein because of loss of the pVHL gene, indicating that HIF-1 α is inhibited independently of the pVHL pathway. He suggests that inhibition of HIF-1 α by Trx-1 inhibitors may contribute to the growth inhibitory and antitumor activity of these agents.

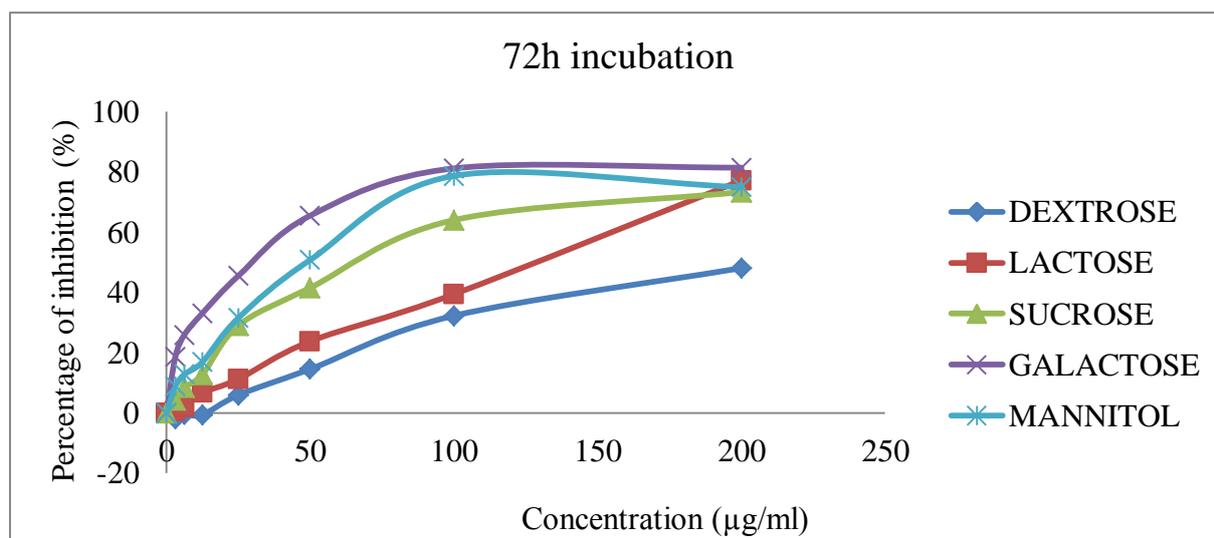


Figure 3- *In vitro* growth inhibitions of MCF-7 cells by pleurotin extracted from liquid media containing different source of sugar (40 g/l) determined by sulforhodamine B (SRB) assay in 72 h incubation period.

Table 1- The IC_{50} values ($\mu\text{g/ml}$) of pleurotin extracted from liquid media with different source sugar tested against MCF-7 breast carcinoma, 72 h incubation period.

Sugar source	IC_{50} values ($\mu\text{g/ml}$)
Galactose	29.84 ± 2.37
Mannitol	48.90 ± 3.74
Sucrose	65.20 ± 3.26
Dextrose	$> 200 \pm 0.00$
Lactose	128.80 ± 9.42
LSD value	36.826^{**}
$** (P \leq 0.01)$.	

Cytotoxic Effect of Pleurotin

The results of cytotoxicity effects of pleurotin and Doxorubicin (positive control) against the CaSki cell line and A549 cell line were summarized in Table-2 while the result of cytotoxicity effects of pleurotin and Cisplatin (positive control) on the MCF-7 cell line and MRC-5 cell line were summarized in Table-3.

In table-3 pleurotin extracted from *Pleurotus ostereatus* (P.11) supplemented with galactose as a source of sugar showed effective cytotoxicity on all three selected cancer cell lines (CaSki, MCF-7 and A549) except non-cancer fibroblast MRC-5 cell line. Pleurotin showed the most effective significantly growth inhibition on breast adenocarcinoma cell line MCF-7 followed by cervical carcinoma cells CaSki ($P \leq 0.05$), lung adenocarcinoma A549 ($P \leq 0.01$) with the IC_{50} values 29.84 ± 2.37 $\mu\text{g/ml}$, 30.25 ± 2.40 $\mu\text{g/ml}$ and 37.60 ± 2.65 $\mu\text{g/ml}$ respectively. Cytotoxicity of pleurotin was compare with cytotoxicity of the Doxorubicin (positive control) as a chemotherapeutic drug on the CaSki cell line with IC_{50} 0.18 ± 0.00 $\mu\text{g/ml}$ and A549 cell line with IC_{50} 1.10 ± 0.02 $\mu\text{g/ml}$, while Cisplatin (positive control) compared with pleurotin on the MCF-7 cell line and MRC-5 cell line with IC_{50} 8.20 ± 0.25 and 8.88 ± 0.13 $\mu\text{g/ml}$ respectively.

In this study pleurotin has a toxic effect on cancer cells while it showed a low effect on the non-cancer fibroblast which means pleurotin it is safety to normal cell and toxic to cancer cell compared with Cisplatin, it has IC_{50} 8.88 ± 0.13 while growth inhibition rate for pleurotin on MRC-5 cell line was less 30% when the pleurotin titer > 200 $\mu\text{g/ml}$.

In spite of that both positive control (Doxorubicin and Cisplatin) had an activity to kill cancer cells with low IC_{50} , but at the same time they have harmful side effects on normal cells. While pleurotin did not recorded any side effects in this study or in any previous studies.

The most dangerous side effect of doxorubicin is cardiomyopathy, leading to congestive heart failure. Another common and potentially fatal complication of doxorubicin is typhilitis, an acute life-threatening infection of the bowel [16]. Additionally, some patients may develop PPE, characterized by skin eruptions on the palms of the hand or soles of the feet, swelling, pain and erythema [17]. It can cause reactivation of hepatitis B, and doxorubicin-containing regimens are no exception. Due to these side effects and its red color, doxorubicin has earned the nickname "red devil" or "red death" [18].

Cisplatin is able to stop proliferation of cancer cells by creating the DNA-platinum. Interaction was discovered and word wide used in clinical medicine. However, its effectiveness has been limited by the occurrence of dose related Nephrotoxicity, Neurotoxicity, blood test abnormalities, low white blood cells and low red blood cells, Nausea and vomiting, Ototoxicity and Hemolytic anemia [19].

Table 2- IC₅₀ values (µg/ml) of Pleurotin and Doxorubicin (positive control) on selected cancer cells, CaSki and A549 lines.

Cells	Type	Incubation Time (Hrs)	Pleurotin IC ₅₀ (µg/ml)	Doxorubicin IC ₅₀ (µg/ml)
CaSki	Cervical carcinoma	72	30.25 ± 2.49	0.18 ± 0.00
A549	Lung adenocarcinoma	72	37.60 ± 2.86	1.10 ± 0.02
LSD value	---	---	5.162 *	0.462 *

* (P≤0.05).

Doxorubicin was use as the reference compound

Table 3- IC₅₀ values (µg/ml) of Pleurotin and Cisplatin (positive control) on selected cancer cells, MCF-7 and MRC-5 cell lines.

Cells	Type	Incubation Time (Hrs)	Pleurotin IC ₅₀ (µg/ml)	Cisplatin IC ₅₀ (µg/ml)
MCF-7	Breast adenocarcinoma	72	29.84 ± 1.73	8.20 ± 0.25
MRC-5	Non cancer fibroblast	72	>200 ± 0.00	8.88 ± 0.13
LSD value	---	---	39.52 **	12.83 **

** (P≤0.01).

Cisplatin was use as the reference compound.

References

1. Al Hasnawi, S. M., Al Mosawi, A. J., Al khzaie, A., Unan, O., F., Fadhil, H. M. and Sami, S. **2009**. Cancer in Iraq: Distribution by primary tumor site. *The New Iraqi Journal of Medicine, Oncology*. 5 (1): 5- 8.
2. Houghton, P., Fang, R., Techatanawat, I., Steventon, G., Hylands, P.G.and Lee, C.C. **2007**. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. *Methods*. 42: 377–387.
3. Husain, H. Y., Al-Alawachi, S. F. A. **2014**. Incidence rates, pattern and time trends of registered cancer in Iraq (1991-2008) population and hospital based registry. *Open Access Library Journal*, 1, P: 1-6.
4. Busby, C., Hamdan, M. and Ariabi, E. **2010**. Cancer, infant mortality and birth sex-ratio in Fallujah, Iraq 2005–2009. *Int. J. Environ. Res. Public Health*, 7, P: 2829- 2837.
5. La Guardia, M., Venturella, G and Venturella, F. **2012**. On the chemical composition and nutritional value of *Pleurotus* taxa growing on umbelliferous plants (Apiaceae). *J. Agr. Food Chem*. 53: 5997 – 6002.
6. Smiderle, F. R., Olsen, L. M., Ruthes, A. C., Czelusniak, P. A., Santana-Filho, A. P., Sasaki, G. L., Gorin, P. A. J. and Iacomini, M. **2012**. Exopolysaccharides, proteins and lipids in *Pleurotus pulmonarius* submerged culture using different carbon sources. *Carbohydrate Polymers*. 87 : 368– 376.
7. Shipley, S. M., Barr, A. L., Graf, S. J., Collins, R. P., McCloud, T. G. and Newman, D. J. **2006**. Development of a process for the production of the anticancer lead compound pleurotin by fermentation of *Hohenbuehelia atrocaerulea*. *J. Ind. Microbiol. Biotechnol*. 33: 463–468.

8. Robbins, W. J., Kavanagh, F. and Hervey, A. **1947**. Antibiotic substances from basidiomycetes I. *Pleurotus griseus*. *Proc Nat Acad Sci. U.S.A. Bot.* 33:171–176.
9. Abdul Rahman, S. N., Abdul Wahab, N. and Abd Malek, S. N. **2013**. In Vitro morphological assessment of apoptosis induced by antiproliferative constituents from the rhizomes of *Curcuma zedoaria*. *Evidence-Based Complementary and Alternative Medicine*. Volume 2013, Article ID 257108, 14 pages.
10. Lee, G. S. **2011**. Bioactivity and chemical investigation of *Curcuma Mangga* and *Curcuma Inodora*. Ph. D. Thesis, Faculty of science, University of Malaya, Kuala Lumpur.
11. Vichai, V. and Kirtikara, K. **2006**. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols*. 1(3): 112-6.
12. Wei, Q. Y., Ma, J. P., Cai, Y. J., Yang, L. and Liu, Z. L. **2005**. Cytotoxic and apoptotic activities of diarylheptanoids and gingerol-related compounds from the rhizome of *Chinese ginger*. *J. Ethnopharm.* 102: 177-184.
13. SAS. **2012**. Statistical analysis system, *User's Guide. Statistical*. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
14. Elsayed, M. A., Hassan, M. M., Elshafei, A. M., Haroun, B. M. and Othman, A. M. **2012**. Optimization of cultural and nutritional parameters for the production of laccase by *Pleurotus ostreatus* ARC280. *British Biotechnology Journal*, 2(3): 115-132.
15. Welsh, S. J., Williams, R. R., Birmingham, A., Newman, D. J., Kirkpatrick, D. L. and Powis, G. **2003**. The Thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and Pleurotin inhibit Hypoxia-induced factor 1 and vascular endothelial growth factor formation. *Molecular Cancer Therapeutics Vol. 2*, 235–243.
16. Kaczmarek, A., Brinkman, B.M., Heyndrickx, L., Vandenabeele, P., Krysko, D. V. J. **2012**. Severity of doxorubicin-induced small intestinal mucositis is regulated by the TLR-2 and TLR-9 pathways. *J. Pathol.* 226 (4): 598–608.
17. Raritan, L.P. **2007**. Product information. DOXIL, *doxorubicin HCL liposome injection*. U.S.A.
18. Groopman, J. E. **2007**. How doctors think. *Boston: Houghton Mifflin*. p. 49. ISBN 0-618-61003-0.
19. Shin, S. K. **2009**. Bioactivity and chemical investigation of *Pereskia biao* and *Pereskia gradifolia*. Ph. D. Thesis. University of Malaya. Kuala Lumpur.