

Anticancer potential of *Hericium erinaceus* extracts against particular human cancer cell lines

Younis AM

Department of Botany and Microbiology, Faculty of Science, Al Azhar University, Cairo, 11844, Egypt - amyounis@mymail.vcu.edu

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Abstract

Cancer is a leading cause of death worldwide. Cancer resulted in 8.2 million human deaths in 2012. It is expected that annual cancer cases will rise from 14 million in 2013 to 22 million within the next two decades. Mushrooms are extensively used as nutritional supplements in many countries. Moreover, mushrooms have many medicinal properties, including anticancer activity. In this study, the anticancer activity of different polar and non-polar extracts of *Hericium erinaceus* were evaluated against different human cancer cell lines including human liver carcinoma (Hep G2), the human colonic epithelial carcinoma (HCT 116), the human cervical cancer cells (HeLa) and the human breast adenocarcinoma (MCF-7) using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Furthermore, as a control, the cytotoxicity effect of the different extracts were tested against isolated mouse hepatocytes. It was observed that the extracts by water and methanol from fresh and lyophilized fruiting bodies of *H. erinaceus* had the strongest anticancer effect. In contrast, the extracts by ether and ethyl acetate from mycelia and broth of *H. erinaceus* showed lower anticancer activity against the tested carcinoma cell lines. The highest anticancer activity was recorded for aqueous extract of lyophilized fruiting bodies with half maximal inhibitory concentration (IC₅₀) values of 6.1±0.2, 5.1±0.1, 5.7±0.2 and 5.8±0.3 µg/ml against Hep G2, HCT 116, HeLa and MCF-7 cells, respectively with non-significant effect on the normal mouse hepatocytes. To summarise, polar extracts of *H. erinaceus* can be good sources for isolating natural anticancer compounds. I recommend further chemical studies to isolate the active principles of the extract of *H. erinaceus* evaluated in the present.

Key words – Anticancer activity – *Hericium erinaceus* – monkey's head mushroom – mushroom extracts.

Introduction

Cancer is a leading cause of death worldwide. Cancer caused 8.2 million human deaths in (World Health Organization (WHO) 2012). By 2050, a 3-fold increase in incident cancer is estimated. The annual financial costs of cancer have been estimated at \$1.16 trillion US dollars per year as of 2012 (WHO 2014).

In the Egyptian context, according to the National Cancer Research Institute (NCRI) and Cancer Index, the chance of getting cancer before age 75 is around 15%. People newly diagnosed with cancer number around 100,000 per year. Deaths from cancer are around 70,000 per year. The most common causes for both sexes together are liver (24%), breast (15%), and

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Corresponding Author: Younis AM – e-mail – amyounis@mymail.vcu.edu

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bladder (7%). For Egyptian men, liver (34%) and bladder (11%) are the most common types while, for women the breast (32%) and liver (14%) as mentioned by various investigators (WHO 2012; Ibrahim 2014).

Treatment options often are expensive and will have side effects. For example, most chemotherapeutic agents for the treatment of cancer not only destroy tumors and stop cancer progress but also have an effect on the healthy cells and tissues. This situation has encouraged many scientists to search for natural products with anticancer activity to be more effective and safer agents to inhibit the growth of cancer cells (Younis *et al.* 2014a).

Experimental studies have demonstrated that many natural products from fungi have anticancer potential in a variety of bioassays systems and animal models (Evidente *et al.* 2014). Many mushrooms can be considered to be food of high nutritional value because they contain high quality proteins and most of the essential amino acids. They are also a good source of unsaturated fat, phosphorus, iron, and vitamins, including thiamine, riboflavin, ascorbic acid, ergosterol and niacin (Kalac 2009). Medicinal mushrooms have been used as traditional medicine for the treatment of various diseases due to their important therapeutic properties included, but not limited to, the anticancer activity (Wasser 2014).

Collectively, many researchers have investigated the antitumor activities of mushrooms and isolated mushroom metabolites with beneficial properties against cancer (Wu *et al.* 2011; Finimundy *et al.* 2013; Younis *et al.* 2014a, b).

Many polysaccharides isolated from mushrooms have strong antitumor activities (Moharib *et al.* 2014); for example, *Lentinan* which is a polysaccharide isolated from *Lentinula edodes* and have strong anticancer activities has been used in the clinic for treating of several cancers, especially stomach cancer (Yamaguchi *et al.* 2011).

Hericium erinaceus has attracted the attention of many researchers due to its anti-cancer, hepatoprotective and immunomodulatory effect (Patel and Goyal 2012). For example, Kim *et al.* (2011) investigated the anti-tumor effects of the *H. erinaceus* extracts in Balb/c mice transplanted with CT-26 colon cancer cells and observed that hot-water of fruiting bodies are rich with β -glucan which reduced tumor weights by 38 % when injected daily for 2 weeks. In addition Li *et al.* (2014) isolated twenty-two compounds from *H. erinaceus* fractions and exhibited *in vitro* antitumor activities against liver cancer HepG2 and Huh-7, colon cancer HT-29, and gastric cancer NCI-87 cell lines and reported *in vivo* a significantly antitumor efficacy against all four xenograft models of HepG2, Huh-7, HT-29 and NCI-87 without toxicity to the host.

In this study, the anticancer activity of *H. erinaceus* extracts were evaluated against different human cancer cell lines using MTT assay. Furthermore, the cytotoxicity effect of *H. erinaceus* extracts were tested against isolated mouse hepatocytes.

Materials and methods

H. erinaceus fruiting bodies and mycelia

The fruiting bodies of *H. erinaceus* were collected from natural growth on trees trunk located at Richmond, Virginia, USA. Mycelia were isolated from the fruiting bodies and transferred to potato dextrose agar (PDA) medium, consisting of 4 g/L potato extract (Sigma-Aldrich, Missouri, USA), 20 g/L dextrose (Pharmacia, New York, USA), 20 g/L agar (Sigma-Aldrich) (Younis *et al.* 2014a).

For liquid culture, the mycelia of *H. erinaceus* were grown in 250 ml Erlenmeyer flasks containing 100 ml of a potato dextrose broth (PDB) medium consisting of 4 g/L potato extract and 20 g/L dextrose, and incubated at 24° C for 15 days. Then the mycelia were separated from the broth by filtration using 0.2 μ m filter, the filtrates were concentrated by lyophilization with a

Virtis BT4KZL-105 lyophilizer (SP Industris, Warminster, PA, USA) and stored at -20° C until use (Younis *et al.* 2015)

Mushroom extracts

The extraction was performed using polar solvent as distilled water and methanol (Sigma-Aldrich), and non-polar solvents ad ether (Sigma-Aldrich) and ethyl acetate (Sigma-Aldrich) on the fresh and lyophilized fruiting bodies as well as mycelia and broth.

The fresh fruiting bodies (FFB) were washed with distilled water, blot dried, cut into pieces, and immersed in the following solvents distilled water, methanol, ether or ethyl acetate at 10g/100ml (wt/v), then stored in the refrigerator at 4°C. After 24 hours the mixture was ground with a Waring commercial laboratory blender (Fisher Scientific, Inc., Waltham, MA, USA), and sonicated in an ultra-sonicator (Fisher Model 300 Sonic Dismembrator- Fisher Scientific, Inc., Waltham, MA, USA) for 30 min at 25 KHz. Then it was centrifuged at 10,000 xG for 20 minutes in (Beckman CS-6R Centrifuge- Beckman Coulter, Inc. Atlanta, GA, USA), then filtrated and the supernatants were collected. The water extract was concentrated by lyophilization. Other extracts were concentrated by air drying and re-suspended in dimethyl sulfoxide (DMSO) (Fisher) (Younis *et al* 2014a).

For the lyophilized fruiting bodies (LFB) the above method was used for the extractions using LFB at 10g/100ml (wt/v)

For mycelia extracts the mycelia were collected and washed with distilled water, blot dried, and the same method above was used for the extractions using LFB at 10g/100ml (wt/v)

For the broth extracts, the lyophilized broth was dissolved in distilled water, methanol, ether and ethyl acetate at 1g/10ml (wt/v). The water extract was concentrated by lyophilization, other extracts were concentrated by air drying and resuspended in DMSO.

Assay of anticancer activity of *H. erinaceus* extracts against the carcinoma cells lines

Four carcinoma cell lines were used to test the anticancer activity of *H. erinaceus* extracts, including human liver carcinoma cells (Hep G2) (ATCC[®] HB-8065[™]) that cause hepatocellular carcinoma, the human colonic epithelial carcinoma (HCT 116) (ATCC[®] CCL-247[™]) that causes colorectal carcinoma, the human cervical cancer cells (HeLa) (ATCC[®] CCL-2[™]) from an adenocarcinoma and the human breast adenocarcinoma cells (MCF-7) (ATCC[®] HTB-22[™]).

The four carcinoma cell lines were incubated in 75 cm² culture corning flask (Fisher Scientific Inc., Loughborough, Leicestershire, United Kingdom) at 37°C in a water jacketed incubator (Forma Scientific series II, (Thermo Scientific Inc., Waltham, MA, USA) using their specific medium reported by American Type Culture Collection (ATCC). An inverted microscope (Olympus, CKX41; Shinjuku, Tokyo, Japan) was used to view the cell monolayer and confirm the absence of any contamination with bacteria and/or fungi. To count the number of cells, the cell monolayer was washed with 5 ml phosphate buffer saline (PBS) without Ca²⁺/Mg²⁺ (Life Technologies) then 2.5 ml of 0.53 mM trypsin/EDTA (Life Technologies) was added to the culture flask, and incubated for 7-15 min. When cells were displaced from the flask, 6 ml of maintenance media were added to stop the action of the trypsin. A hemocytometer was used to determine the number of viable cells using trypan blue staining (Life Technologies) (Younis *et al.* 2014b).

For anticancer assays, cells were suspended in medium at concentration 5x10⁴ cell/well in 200 µl/well in Corning 96-well tissue culture plates (Fisher Scientific Inc., UK), then incubated for 24 hr. Extracts were then dispensed in 50 µl volumes into 96-well plates with six concentrations for each extract = 50, 25, 12.5, 6.25, 3.125, 1.56 µg/ml, and each concentration was repeated 5 times. Control with media or 0.5 % DMSO were run for each 96 well plate. After incubating for 24 h, the numbers of viable cells were determined by the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich) test. This test was

conducted as follows: the media was removed from the 96 well plate and replaced with 100 μ l of fresh culture RPMI 1640 medium without phenol red (Life Technologies) then 10 μ l of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO₂ for 4 hours. Then an 85 μ l aliquot of the media was removed from the wells, and 50 μ l of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. The optical density was measured at 590 nm with the micro plate reader (Bio-TekuQuantmicroplate spectrophotometer BioTek, inc, Winooski, VT. USA). The anticancer activity was determined by the mean percent inhibition of the tumor cells remaining after the treatment by the following formula: (ODt/ODc) x100% where ODt and ODc are the optical densities of wells with treated and untreated cells, respectively (Wu and Wang 2010; Elaasser *et al.* 2011; Younis *et al.* 2014b). The half maximal inhibitory concentration (IC₅₀) was determined after plotting the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA).

Cytotoxicity assay

H. erinaceus extracts were tested for cell toxicity using isolated normal mouse hepatocytes by a novel technique adopted by the author after Li *et al.* (2010). 21 g female mouse (*Mus musculus* C57/BL6) was euthanized by carbon dioxide for 1 min, after death the mouse was placed onto a sterile tray and the fur on the abdomen was sprayed with a sterilized by 70% ethanol. Then a sterile lancet was used to cut the abdomen skin. The intestines moved to the right to uncover the portal vein and the vena cava. Then a 21 G vein infusion set (Traverol laboratories Inc., Deerfield, IL. USA) inserted into the vena cava. The perfusion medium (I) consisting of 500 ml sterile (PBS), 5 ml of 1 M HEPES in 5% (w/v) KCl buffer (pH 7.4), 2.5 ml 1 M of sterile and filtered glucose, 0.5 ml 200 mM EDTA (ethylene diamine tetra acetate), 0.5 ml phenol red solution, pH adjusted to 7.4, were injected into the vena cava to perfuse through the liver. Finally the portal vein was cut to allow the blood to get out. The perfusion medium I was used for 10–15 min till the liver became clear of blood and turned white. Then the collagenase solution was used for approximately 10 min. The entire liver was removed to a sterile petri dish containing perfusion medium (II) consisting of 500 ml sterile PBS, 5 ml 1 M HEPES in 5% (w/v) KCl buffer (pH 7.4), 10 ml 1 M HEPES, 2.5 ml 1 M glucose (sterile filtered), 1 ml 500 mM CaCl₂, 0.5 ml phenol red solution, pH adjusted to 7.4, and the liver was cut into small pieces using two sterile pairs of forceps. The crude hepatocyte, in the perfusion medium (II), was then filtered through a gauze mesh filter (100 μ m in diameter) and transferred the resulting cell suspension into 50 ml sterile tubes and centrifuged at 1050 rpm for 2 min. The supernatant discarded and the parenchymal hepatocytes were washed three times using the same procedure by resuspending the pellet each time in fresh perfusion medium II and centrifuging at 1050 rpm for 2 min. Then the hepatocytes were resuspended in 30 ml Williams E culture medium consisting of Williams E medium with L-glutamine, supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1% (v/v) pen/step, 1% (v/v) nonessential amino acids and 2.2 g/L sodium bicarbonate. The hepatocytes were checked for cell numbers and viability using trypan blue and a hemocytometer (Li *et al.* 2010). The isolated normal mouse hepatocytes were suspended in medium at concentration 5×10^4 cell/well and 200 μ l/well dispensed into Corning® 96-well tissue culture plates then incubated for 24 hr at 37 °C in a water jacketed double door incubator under 5% CO₂. After the inoculation, the cells were viewed using an inverted microscope to view the cell viability and confirmed the absence of any contamination with bacteria and/or fungi and now ready for the cytotoxicity assay.

For each *H. erinaceus* extract, six different concentrations (five replica each) of 50, 25, 12.5, 6.25, 3.125, 1.56 μ g/ml were prepared, and then dispensed into 96-well plates at 50 μ l per well. The 96-well plates were incubated at 37°C in a humidified incubator with 5% CO₂ for a period of 24 h with a control of untreated cells. The numbers of viable cells were determined by

MTT as previously mentioned before and the percentage of inhibition was calculated as $[1-(OD_t/OD_c)] \times 100\%$ where OD_t is the mean optical density of wells treated with the tested compounds and OD_c is the mean optical density of untreated cells (Li *et al.* 2010).

Results

The anticancer activity of *H. erinaceus* extracts against the four carcinoma cell lines and the cytotoxicity of these extracts against the isolated normal mouse hepatocytes is displayed in Table 1. The highest anticancer effect was reported by the aqueous extract of lyophilized fruiting bodies with maximal inhibition percentage (MPI) of 92 ± 2.1 , 96.1 ± 1.5 , 94.2 ± 2.6 and 95.5 ± 2.3 % with half maximal inhibitory concentration (IC_{50}) values of 6.1 ± 0.2 , 5.1 ± 0.1 , 5.7 ± 0.2 and 5.8 ± 0.3 $\mu\text{g/ml}$ against Hep G2, HCT 116, HeLa and MCF-7 cells, respectively with non-significant effect (MPI = 6.6 ± 0.8) % on the normal mouse hepatocytes 6.6 ± 0.8 .

Also, the methanol of LFB showed high anticancer effect with MPI of 85.1 ± 2.7 , 87.3 ± 2.8 , 86.2 ± 2.9 and 83.1 ± 1.9 % and IC_{50} of 15.1 ± 1.7 , 9.7 ± 0.8 , 11.1 ± 0.8 , and 8.5 ± 0.9 $\mu\text{g/ml}$ against Hep G2, HCT 116 HeLa and MCF-7 cells, respectively. Followed by the aqueous of FFB which showed high MPI of 83 ± 2.1 , 75.8 ± 1.2 , 79.4 ± 2.5 and 77.1 ± 1.9 % with IC_{50} of 7.9 ± 0.9 , 11.7 ± 0.9 , 10.7 ± 1.8 and 12.5 ± 1.9 $\mu\text{g/ml}$ against Hep G2, HCT 116, HeLa and MCF-7 cells, respectively. In addition, both methanol extract of LFB and aqueous extract of FFB reported low cytotoxicity effect against the isolated mouse hepatocytes with MPI 11.3 ± 1.1 and 8.3 ± 1.1 % respectively (Table 1, Figures 1-5).

Furthermore, the methanol extract of FFB reported high MPI against the four carcinoma cells and low MPI against normal mouse hepatocytes with MPI of 74.7 ± 1.4 , 71.1 ± 2.1 , 64.9 ± 1.2 , 68.8 ± 2.1 and 12.5 ± 1.9 % against Hep G2, HCT 116 HeLa, MCF-7 and normal mouse hepatocytes, respectively (Table 1, Figures 1-5).

In comparison, ether and ethyl acetate extracts showed less effect than the aqueous and methanol extracts against the four carcinoma cells. The ether and ethyl acetate of both FFB and LFB had approximately the same ranges of MPI (43-33 %) and IC_{50} (60-91 $\mu\text{g/ml}$) against most tumor cell lines and approximately the highest effect reported in this study against the isolated normal mouse hepatocytes with MPI ranges between (32-29) %.

In contrast, extracts from mycelia and broth showed lower anticancer activities than extracts from FFB and LFB. the highest effect was observed by the aqueous extract of mycelia and showed MPI of 67.6 ± 3.2 , 73.5 ± 1.8 , 71.3 ± 1.9 and 76.6 ± 3.2 % and IC_{50} of 31.2 ± 2.2 , 29.9 ± 1.7 , 28.8 ± 1.8 and 25.2 ± 2.2 $\mu\text{g/ml}$ against Hep G2, HCT 116 HeLa and MCF-7 cells, respectively. Non-significant effect with 10.5 % MPI was observed against the normal mouse hepatocytes.

Both aqueous extract of broth and methanol extract of mycelia or broth showed approximately the same MPI ranges between (53-43) % with IC_{50} ranged between (53-43) $\mu\text{g/ml}$. the effect of these extracts on the isolated normal mouse hepatocytes was varied and showed 7.1 % by water extract of broth and 14.6 % by methanol extract of broth (Table 1, Figures 1-5).

Finally, the ether and ethyl acetate extracts from mycelia and broth showed low effect on the four carcinoma cells and higher effect against normal mouse hepatocytes.

The lowest effect observed by the EE of broth with MPI of 7.2 ± 0.2 % against HeLa cells with estimated IC_{50} 336 ± 5.1 (Table 1).

Table 1 The anticancer activities of *H. erinaceus* extracts against the four carcinoma cell lines and the cytotoxicity effect against the isolated normal mouse hepatocyte

Sample	Solvent	HepG2		HCT 116		HeLa		MCF-7		Normal mouse hepatocyte
		Maximum inhibitory %	IC ₅₀ (µg/ml)	Maximum inhibitory %	IC ₅₀ (µg/ml)	Maximum inhibitory %	IC ₅₀ (µg/ml)	Maximum inhibitory %	IC ₅₀ (µg/ml)	Maximum inhibitory %
Fresh fruiting bodies	Water	83±2.1	7.9±0.9	75.8±1.2	11.7±0.9	79.4±2.5	10.7±1.8	77.1±1.9	12.5±0.9	8.3±1.1
	Methanol	74.7±1.4	15.1±1.3	71.1±2.1	21.8±1.7	64.9±1.2	26.1±2.1	68.8±2.1	24.3±2.4	12.5±1.9
	Ether	33.1±2.8	86.7±3.8	35.3±2.1	73.1±2.4	33.7±2.6	91.2±2.3	40.7±2.2	81.3±2.6	32.3±2.2
	Ethyl Acetate	35±1.7	69.1±3.1	34.6±1.8	64.2±2.3	43.4±2.5	65.6±2.3	39.3±2.0	62.9±2.3	29.6±2.7
Lyophilized fruiting bodies	Water	92±2.1	6.1±0.2	96.1±1.5	5.1±0.1	94.2±2.6	5.7±0.2	95.5±2.3	5.8±0.3	6.6±0.8
	Methanol	85.1±2.7	15.1±1.7	87.3±2.8	9.7±0.8	86.2±2.9	11.1±0.8	83.1±1.9	8.5±0.9	11.3±1.1
	Ether	42.2±1.2	68.5±2.5	41.1±2.5	67.4±2.3	43.2±2.2	60±1.7	37.8±1.9	68.5±2.6	23.2±1.2
	Ethyl Acetate	33.9±1.5	63.9±3.5	37.6±2.9	63.1±2.8	39.6±2.2	63.7±2.3	43.6±1.9	56.6±1.7	21.1±2.1
Mycelia	Water	67.6±3.2	31.2±2.2	73.5±1.8	29.9±1.7	71.3±1.9	28.8±1.8	76.6±3.2	25.2±2.2	10.5±0.9
	Methanol	50.9±1.9	45.9±1.9	52±2.5	50.9±2.1	53±2.4	42.9±2.1	53.2±3.5	44.1±2.5	13.8±2.1
	Ether	21.2±1.6	97.5±4.7	31±1.9	87.5±1.8	26.1±1.2	128±4.1	21.2±1.6	117.5±4.7	31±1.7
	Ethyl Acetate	28.2±1.5	103.2±2.3	34.1±2.1	99.1±2.3	25±1.4	125±3.3	30.2±1.5	93.2±4.3	29.1±2.1
Broth	Water	52.2±1.2	48.5±2.1	44.1±2.5	55.4±2.3	43.2±2.2	50±3.7	43.2±1.2	52.5±2.5	7.1±0.5
	Methanol	43.9±1.5	53.9±3.5	43.6±2.9	53.1±2.1	49.6±2.2	43.7±1.3	49.9±1.5	50.9±3.5	14.6±0.9
	Ether	13.2±1.2	272±3.5	21.3±1.1	179.5±2.9	7.2±0.2	336±5.1	22.7±2.1	127.3±2.8	19.2±1.2
	Ethyl Acetate	25.1±2.5	372±7.1	20±1.2	177.4±2.7	17.2±0.9	196±3.2	24.8±2.1	152.8±2.3	28.1±2.1

The data are expressed as the mean values ± SD; The maximal inhibition percentage of tumor cells determined with 50 µg/ml of each extract. IC₅₀ was determined from a range of concentrations shown in Figures 1-5 using Graphpad Prism software.

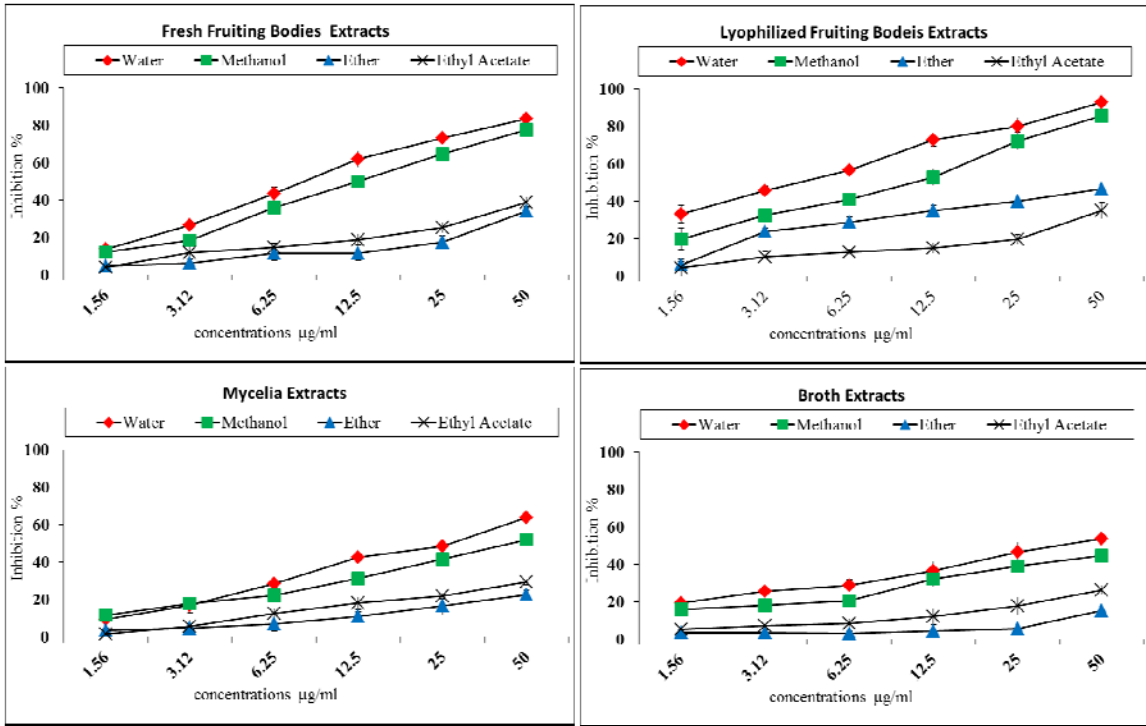


Fig. 1- The anticancer activity of *H. erinaceus* extracts against Hep G2 cell line showing the effect of extract concentrations tested at 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml.

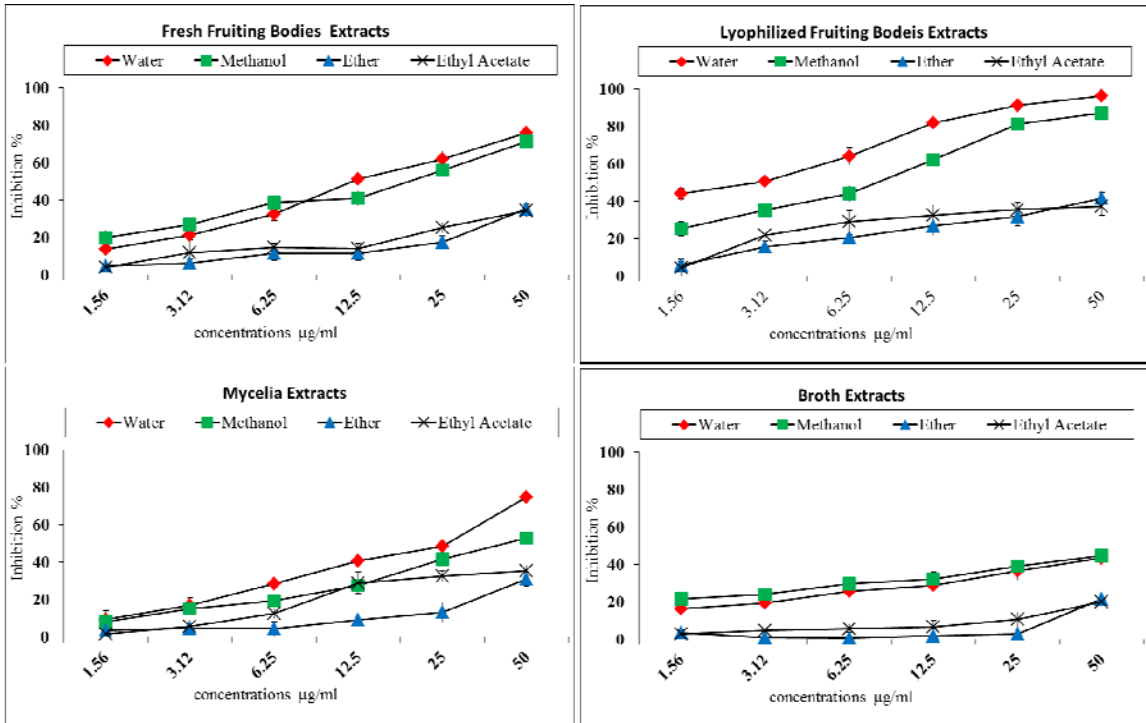


Fig. 2- The anticancer activity of *H. erinaceus* extracts against HCT 116 cell line showing the effect of extract concentrations tested at 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml.

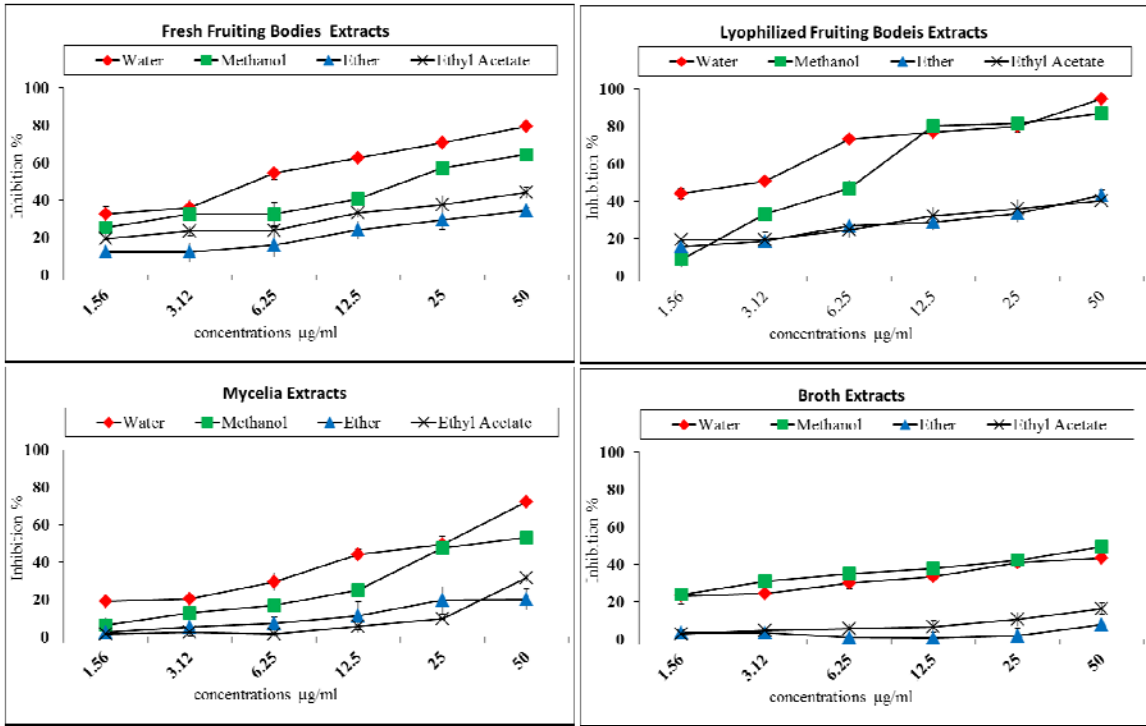


Fig. 3- The anticancer activity of *H. erinaceus* extracts against HeLa cell line showing the effect of extract concentrations tested at 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml.

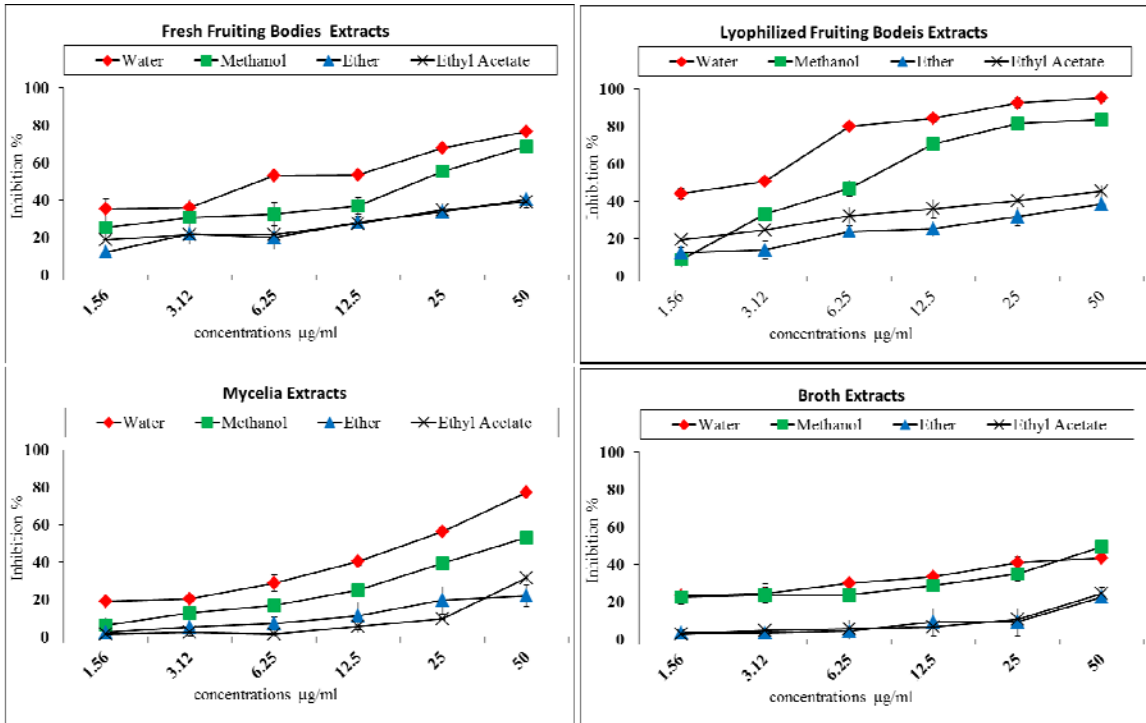


Fig. 4- The anticancer activity of *H. erinaceus* extracts against MCF-7 cell line showing the effect of extract concentrations tested at 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml.

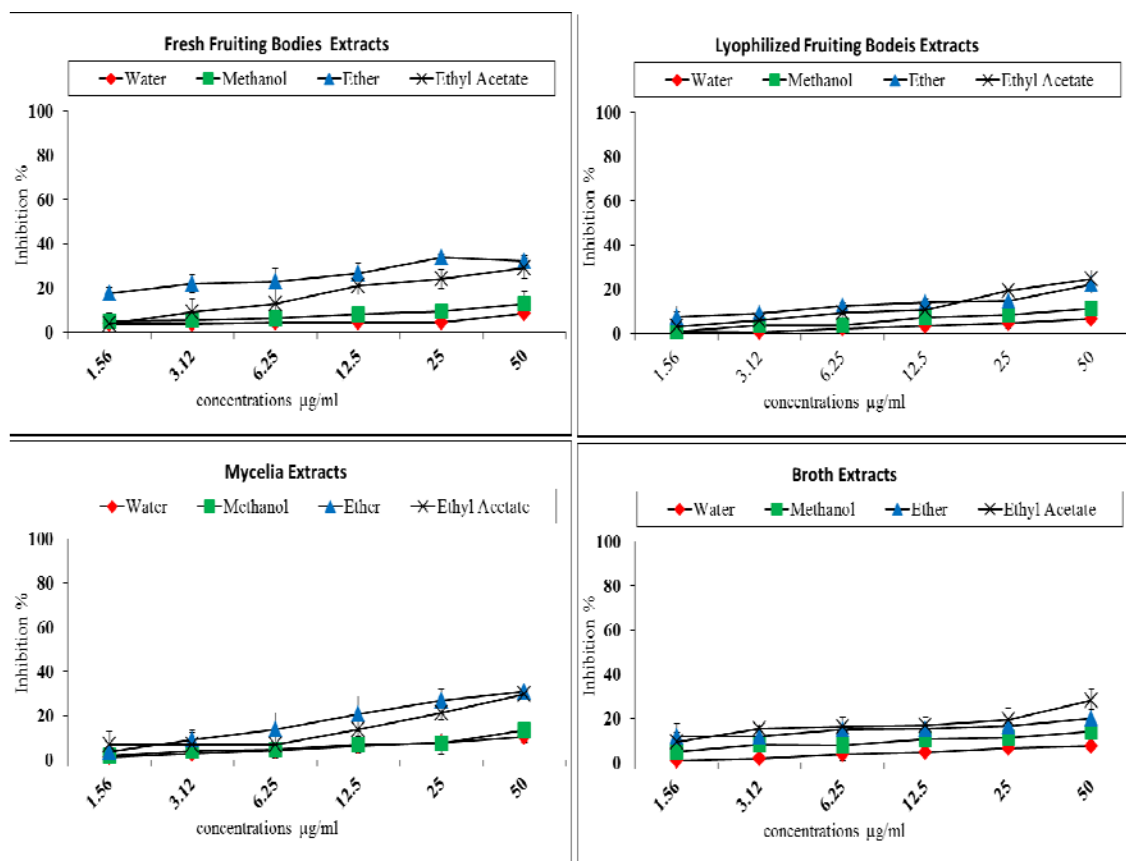


Fig. 5- The cytotoxicity effect of *H. erinaceus* extracts against isolated mouse hepatocytes showing the effect of extract concentrations tested at 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml.

Discussion

Cancer, also known as malignancy, is an uncontrolled growth of cells. There are more than 100 types of cancer, including breast, skin, lung, colon, prostate, and lymphoma cancer. Unfortunately cancer treatment often includes one or more of chemotherapy, radiation and surgery. These treatment options are relatively expensive, unstable, and sometimes have undesirable pharmacological actions and limited range activities (Younis *et al.* 2014a). Medicines from the natural sources usually have lower side effects. Natural products have been the source of a majority of therapeutic agents in modern medicine (Newman 2007). Collectively, many natural products used as important sources of anti-cancer lead molecules. Moreover, many successful anti-cancer drugs are natural products or their analogues. Many more are under clinical trials (Mondal *et al.* 2012).

In this study, different *H. erinaceus* extracts was tested for their anticancer activity against different human cancer cell lines and tested for their cytotoxicity effect against isolated mouse hepatocytes. Results demonstrated that *H. erinaceus* extracts can be a good source for anticancer substances, and these results are consistent with reports indicating that mushrooms extracts had anticancer effects. For example, low-molecular weight substances isolated from mushrooms have been studied and reported to have anticancer activates (Petrova 2012).

In recent years, much attention has been focused on polysaccharides isolated from mushrooms (Jwanny *et al.* 2002; Moharib *et al.* 2014; Yamaguchi *et al.* 2011). In this study, it

was observed that LFB extracts were the most potent extracts with anticancer activity followed by the FFB extracts. In Contrast, the extracts from mycelia and broth had lower anticancer activities than the LFB and FFB extracts of *H. erinaceus* against the four carcinoma cell lines.

Numerous studies have reported the potential medicinal values or health beneficial activities of *H. erinaceus*, like anti-cancer, anti-hypertensive, hypolipidemic, neuronal disease protecting activities (Khan *et al.* 2013). Other researchers demonstrated that *H. erinaceus* polysaccharides possess anti-cancer activities.

Lee and Hong (2010) demonstrated that *H. erinaceus* polysaccharides act as an enhancer to sensitize doxorubicin (Dox)-mediated apoptotic signaling and suggested that *H. erinaceus* polysaccharides in combination with Dox serves as an effective tool for treating drug-resistant human hepatocellular carcinoma.

In this study, the water extract of LFB showed the highest anticancer effect with MPI of 92 ± 2.1 , 96.1 ± 1.5 , 94.2 ± 2.6 and 95.5 ± 2.3 % with IC₅₀ values of 6.1 ± 0.2 , 5.1 ± 0.1 , 5.7 ± 0.2 and 5.8 ± 0.3 µg/ml against Hep G2, HCT 116, HeLa and MCF-7 cells, respectively and showed non-significant effect on the normal mouse hepatocytes 6.6 ± 0.8 %. This result is in accordance with results reported by Lee *et al.* (2009) who found that the crude water-soluble polysaccharide of *H. erinaceus* with anticancer activity due to it up-regulated some functional immuno-modulating events mediated by activated macrophages.

Furthermore, polar solvent extracts showed non-significant effect on the normal mouse hepatocytes, the water extracts of all parts of *H. erinaceus* showed the lowest cytotoxicity effect against the isolated normal mouse hepatocytes with MPI ranged between (6.6-10.5) % followed by the methanol extract with MPI (11.3-14.6) %. On the other hand, non-polar solvent extracts reported higher cytotoxicity against the isolated normal mouse hepatocytes with MPI ranged between (19-32) %.

This result is in accordance with previous study reported that water extract of LFB of *H. erinaceus* exhibited hepatoprotective effect against ethanol-induced gastric ulcers in rats (Abdulla *et al.* 2008). In addition, Zhang *et al.* (2012) reported that the ethanoic extract of endopolysaccharides from *H. erinaceus* mycelia grown on tofu had antioxidant potential and hepatoprotective activity.

This hepatoprotective effect may be due to its high antioxidant capacity. Therefore The *H. erinaceus* polysaccharides could be used as antioxidant product and a supplement in the prevention of hepatic diseases. Also Hao *et al.* (2015) demonstrated that that the aqueous extract of *H. erinaceus* had a relatively significant effect on the hepatic histopathological observations in mice model, which had alcoholic liver damage.

The **conclusion** based on the data available for this study is that *H. erinaceus* extracts may well represent a practical and promising approach for cancer prevention and cancer treatment with non-significant effect on the isolated mouse hepatocytes which gives the possibility of using the *H. erinaceus* extracts as anticancer and hepatoprotective drugs. Epidemiological, histopathological and clinical studies need to be carried out to identify other molecular targets; resolve the relationships between *H. erinaceus* extracts intake and cancer risks; and determine the optimum dosing, efficacy, and safety alone and in combination with chemotherapy and/or radiotherapy. Additionally, more chemical studies will be needed to isolate and identify the active principles of the extract of *H. erinaceus* evaluated in the present. I believe that it is worthwhile to exploit the potential of these antitumor compounds in treating the cancer diseases.

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References

- Abdulla M. A. Noor S. M., Sabaratnam V., Abdullah N., Wong K.H. and Ali H. M. 2008. Effect of culinary-medicinal lion's mane mushroom *Hericiium erinaceus* (Bull.: Fr.) Pers. (Aphyllorphomycetidae), on ethanol-induced gastric ulcers in rats. *International Journal of Medicinal mushrooms* 10(4):325–330.
- Elaasser M. M., Abdel-Aziz M. M. and El-Kassas R. A. 2011. Antioxidant, antimicrobial, antiviral and antitumor activities of pyranone derivative obtained from *Aspergillus candidus*. *Journal of Microbiology and Biotechnology Research* 1:5-17.
- Evidente A., Kornienko A., Cimmino A., Andolfi A., Lefranc F., Mathieud V. and Kiss R. 2014. Fungal metabolites with anticancer activity. *Natural Product Reports* 31:617-627.
- Finimundy T. C., Gambato G., Fontana R., Camassola M., Salvador M., Moura S., Hess J., Henriques J. A., Dillon A. J. and Roesch-Ely M. 2013. Aqueous extracts of *Lentinula edodes* and *Pleurotus sajor-caju* exhibit high antioxidant capability and promising in vitro antitumor activity. *Nutrition Research*, 33:76-84.
- Hao L., Xie Y., Wu G., Cheng A., Liu X., Zheng R., Huo H. and Zhang J. 2015. Protective effect of *Hericiium erinaceus* on alcohol induced hepatotoxicity in mice. *Evidence-Based Complementary and Alternative Medicine* article ID:418023.
- Ibrahim A., Khaled H., Mikhail N., Baraka H. and Kamel H. 2014. Cancer Incidence in Egypt: Results of the National Population-Based Cancer Registry Program. *Journal of Cancer Epidemiology* article ID 437971, doi:10.1155/2014/437971.
- Jwanny E. W., Esmat A. Y., Rashad M. M., Daba A. S. and Abdel-Fattah M. M. 2002. Antitumor activity of polysaccharides extracted from *Pleurotus ostreatus* fruiting bodies and mycelia cultivated on date waste. *The Egyptian Journal of Biochemistry and Molecular Biology* 20:23-40.
- Kalac P. 2009. Chemical composition and nutritional value of European species of wild growing mushrooms. *Food Chemistry* 113: 9-16.
- Khan A. M., Tania M., Liu R. and Rahman M. M. 2013. *Hericiium erinaceus*: an edible mushroom with medicinal values. *Journal of Complementary and Integrative Medicine* 10(1): 1–6
- Kim S. P., Kang M. Y., Kim J. H., Nam S. H. and Friedman M. 2011. Composition and mechanism of antitumor effects of *Hericiium erinaceus* mushroom extracts in tumor-bearing mice. *Journal of Agriculture and Food Chemistry* 59(18):9861-9.
- Lee J. S. and Hong E. K. 2010. *Hericiium erinaceus* enhances doxorubicin-induced apoptosis in human hepatocellular carcinoma cells. *Cancer Letter*, 297(2):144-54.
- Lee J. S., Min K. M., Cho J. Y. and Hong E. K. 2009. Study of macrophage activation and structural characteristics of purified polysaccharides from the fruiting body of *Hericiium erinaceus*. *Journal of Microbiology and Biotechnology* 19:951–9.
- Li G., Yu K., Li F., Xu K., Li J., He S., Cao S. and Tan G. 2014. Anticancer potential of *Hericiium erinaceus* extracts against human gastrointestinal cancers. *Journal of Ethnopharmacology* 153(2):521-30.
- Li W. K., Ralphs L. and Tosh D. 2010. Isolation and Culture of Adult Mouse Hepatocytes. *Book Mouse Cell Culture, Methods in Molecular Biology*. Chapter 13:185-195

- Moharib S. A., Maksoud N. A., Ragab H. M. and Shehata M. M. 2014. Anticancer activities of mushroom polysaccharides on chemically-induced colorectal cancer in rats. *Journal of Applied Pharmaceutical Science* 4: 54-63.
- Mondal S., Bandyopadhyay S., Ghosh M. K., Mukhopadhyay S., Roy S. and Mandal C. 2012. Natural products: promising resources for cancer drug discovery. *Anticancer Agents in Medical Chemistry* 12(1):49-75.
- Newman D. J. and Cragg G. M. 2007. Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products* 70(3):461-77.
- Patel S. and Goyal A. 2012. Recent developments in mushrooms as anti-cancer therapeutics: a review 3 *Biotech* 2(1): 1-15.
- Petrova R.D. 2012. New scientific approaches to cancer treatment: can medicinal mushrooms defeat the curse of the century? *International Journal of Medicinal Mushrooms* 14:1-20.
- Wasser S.P. 2014. Medicinal mushroom science: Current perspectives, advances, evidences, and challenges. *Biomedical Journal* 37(6):345-56.
- WHO. 2012. World Health Organization: Globocan 2012 estimated incidence, mortality and prevalence worldwide in 2012; WHO Press, Geneva, Switzerland, 2- 6.
- WHO. 2014. World Health Organization: World Cancer Report 2014. WHO Press, Geneva, Switzerland, Chapter 1.1, 4.7, 5.12 and 5.14.
- Wu J.Y., Chen C. H., Chang W. H., Chung K. T., Liu Y. W., Lu F. J. and Chen C. H. 2011. Anti-cancer effects of protein extracts from *Calvatia lilacina*, *Pleurotus ostreatus* and *Volvariella volvacea*. *Evidence-Based Complementary and Alternative Medicine* 2011:1-10.
- Wu Y. and Wang Y. 2010. Inhibition of *Astragalus membranaceus* polysaccharides against liver cancer cell Hep G2. *African Journal of Microbiology Research* 4:2181-2183.
- Yamaguchi Y., Miyahara E. and Hihara J. 2011. Efficacy and safety of orally administered *Lentinula edodes* mycelia extract for patients undergoing cancer chemotherapy: a pilot study. *American Journal of Chinese Medicine* 39: 451–9.
- Younis A. M., Wu F. S. and El Shikh H.H. 2015. Antimicrobial activity of extracts of the oyster culinary medicinal mushroom *Pleurotus ostreatus* (Higher Basidiomycetes) and identification of a new antimicrobial compound. *International Journal of Medicinal mushrooms* 17(6):579-90.
- Younis A. Stewart J., Wu F. S., El Shikh H., Hassan F. and Elaasser M. 2014a. Effectiveness of different solvents extracts from edible mushrooms in inhibiting the growth of tumor cells. *Cancer Biology* 4(4): 1-15.
- Younis, A. Stewart, J., Wu F.S., El Shikh H., Hassan F. and Elaasser, M. (2014b) Cytotoxic activity of edible mushrooms extracts against tumor cell lines. *The International Journal of Science and Technology* 3 (11): 736-749.
- Zhang Z., Lv G., Pan H., Pandey A., He W. and Fan L. 2012. Antioxidant and hepatoprotective potential of endo-polysaccharides from *Hericium erinaceus* grown on tofu whey. *International Journal of Biological Macromolecules* 51(5):1140-6.