Effectiveness of different solvents extracts from edible mushrooms in inhibiting the growth of tumor cells

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Abstract: Edible mushrooms have become so bountiful and nourishing as they are a valuable source of nutritional ingredients and biologically active compounds. Moreover, edible mushroom have many medicinal properties, including antitumor activity. In this study, we extracted various parts of three edible mushrooms including *Flammulina velutipes*, *Ganoderma lucidum* and *Pleurotus eryngii* by different polar and non-polar solvents and tested the effectiveness of these extracts in inhibiting the growth of three carcinoma cell lines including human liver carcinoma (Hep G2), the human colonic epithelial carcinoma (HCT 116) and the human cervical cancer cells (HeLa) using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. We observed that the extracts of fresh and lyophilized fruiting bodies had a stronger antitumor effect than extracts from mycelia and broth when extracted by water and methanol. The highest antitumor activity was by the water extract of *F. velutipes* lyophilized fruiting bodies with half maximal inhibitory concentration (IC_{50}) values of 7.3±0.7, 6.9±0.5and 5.2±0.4 µg/ml against Hep G2, HCT 116 and HeLa cells, respectively. Also water extracts of *G. lucidum* lyophilized fruiting bodies showed high antitumor effect with IC_{50} of 10.5±1.1, 7.9±0.6 and 21.4±2.1 µg /ml against Hep G2, HCT 116 and HeLa cells, respectively. The lowest effect was observed by ether extract of *F. velutipes* broth with maximal inhibitory percentage of 5.1±0.8% against HCT 116 cells. In summary, common edible mushrooms may be a source of antitumor compounds.
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1. Introduction

Cancer also known as a malignant tumor is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Cancer is a leading cause of death worldwide resulting in 8.2 million or 14.6% of all human deaths in 2012 (Jemal et al., 2011; Lozano, 2012). Lung, liver, stomach, colorectal and breast cancers cause the most cancer deaths each year. Tobacco use is the cause of about 22% of cancer deaths. In the developing world nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C, and human papillomavirus. The financial costs of cancer have been estimated at $1.16 trillion US dollars per year as of 2010 (Kushi*et* et al., 2012; de Martel et al., 2012; WHO, 2014).

Many scientists search for new antitumor substances from various natural sources to develop more effective and safer agents to inhibit the growth of cancer cells. Natural products from bacteria, fungi and plants have played a leading role in cancer drug discovery resulting in a large number of clinically useful agents (Cragg and Newman, 2005; Chung et al., 2010; Syam et al., 2011; Ren et al., 2012; Bladt et al., 2013; Zhao et al., 2013; Evidente et al., 2014). Experimental studies demonstrated that many naturally occurring agents have anticancer potential in a variety of bioassays systems and animal models, having relevance to human diseases (Sun, 2011).

Many species of mushrooms are not toxic and have been used for food consumption since ancient times (Kalac, 2009). Mushrooms contain highly valued nutritional ingredients (Vaz et al., 2011; Atri et al., 2013; Boda et al., 2012; Lau et al., 2013), as well as medicinal ingredients with medical activities including antimicrobial (Alves et al., 2012;

Chang and Wasser, 2012; Krishnaveni and Manikandan, 2014); antiviral (Pan et al., 2013); antioxidant (Ferreira et al., 2009; Liu et al., 2013; Stajic et al., 2013); anti-hypertensive (Ebigwai et al., 2012) cholesterol-lowering, cardiovascular diseases preventative (Berger et al., 2004; Guillamon et al., 2010; Sato et al., 2013); liver protective (Wu et al., 2013); anti-fibrotic (Iwalokun et al., 2007; Soares et al., 2013); anti-inflammatory (Ganeshpurkar and Rai, 2013; Gunawardenaet al., 2013); anti-diabetic (Lo and Wasser, 2011) and antitumor (Ren et al., 2012; Fontana et al., 2014; Xu et al., 2014) activities.

There are many other examples of isolated mushroom compounds with beneficial properties against cancer (Ferreira et al., 2010; Wu et al., 2011; Finimundy et al., 2013). Many polysaccharides isolated from mushrooms have strong antitumor activities (Zhang et al.,2007; Moharib et al.,2014); for example, Lentinan a polysaccharide isolated from of *Lentinula edodes* showed strong antitumor activities and had been used in the clinic for treating of several cancers, especially stomach cancer (Ng and Yap, 2002; Yamaguchi et al., 2011). In addition, mushroom-derived glucans showed anti-inflammatory and anticancer effects (Schwartz and Hadar, 2014).

In recent years, much attention has been focused on polysaccharides isolated from natural sources such as bacteria, fungi, algae and plants (Jwanny et al., 2009; Sun, 2011). The hot water extracts of five commonly consumed mushrooms of maitake (*Grifola frondosa*), crimini (*Agaricus bisporus*), portabella (*A. bisporus*), oyster (*P. ostreatus*) and white button (*A. bisporus*) significantly suppressed cellular proliferation of MCF-7 cells suggesting common mushrooms may be chemo protective against breast cancer (Martin and Brophy, 2010).

In this study, we investigated the antitumor activity in extracts of several mushroom species against three tumor cell lines. Both polar and nonpolar solvents were used to extract active compounds.

2. Materials and Methods:

2.1. Mushroom strain and culture conditions:

The three edible mushrooms used in this study including *F. velutipes, G. lucidum* and *P. eryngii,* both *F. velutipes* and *P. eryngii* were kindly obtained from the Agriculture Research Center, Cairo, Egypt, and cultivated on rice stew. While, fruiting bodies of *G. lucidum* 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), 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).

For liquid culture, the mycelia of the three mushroom strains were grown in 250 ml Erlenmyer 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. To separate the mycelia from the broth, the cultures were filtered with 0.2 µm filter, and the filtrates were concentrated by lyophilization with a Virtis BT4KZL-105 lyophilizer (SP Industris, Warminster, PA, USA) and stored at -20° C until use.

2.2. Mushroom extracts:

The extraction was performed with distilled water, methanol (Sigma-Aldrich), ether (Sigma-Aldrich) and ethyl acetate (Sigma-Aldrich) on the fresh and lyophilized fruiting bodies as well as mycelia and broth of the three mushrooms growing on PDB.

Fresh fruiting bodies:

The fresh fruiting bodies were washed with distilled water, blot dried, cut into pieces (1x1 cm approximately), and added into the following solvents at 10g/100ml (wt/v): distilled water, methanol, ether or ethyl acetate. The samples then soaked overnight at 4° C and ground with a Waring commercial laboratory blender (Fisher Scientific, Inc.,Waltham, MA, USA), On the second day, the samples were sonicated in an ultra-sonicator (Fisher Model 300 Sonic Dismembrator- Fisher Scientific, Inc., Waltham, MA, USA) for 30 min at 25 KHz. The mixtures were centrifuged (Beckman CS-6R Centrifuge- Beckman Coulter, Inc. Atlanta, GA, USA) at 10,000 x*G* for 20 minutes, and supernatants were collected. The water extract was concentrated by lyophilization with a Labconco FreeZone Benchtop Freeze Dry System (Kansas City, MO- USA). Other extracts were concentrated by air drying and resuspended in dimethyl sulfoxide (DMSO) (Fisher).

Lyophilized fruiting bodies:

The lyophilized fruiting bodies were weighted and added into the following solvents at 10g/100ml (wt/v): distilled water, methanol, ether or ethyl acetate. Then the above method was used for the extractions.

Mycelia and broth:

The mycelia of the three mushrooms were grown on PDB medium, collected and washed with distilled water, blot dried, and added into the following solvents at 10g/100ml (wt/v): distilled water, methanol, ether or ethyl acetate. The samples were then soaked overnight in at 4° C and ground by a blender. On the second day the samples are sonicated with the ultra-sonicator for 30 min at 25 KHz.

The mixtures were centrifuged at 10,000 x *G* for 20 minutes, and the supernatants were collected. Also, 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 dimethyl sulfoxide.

2.3- Assay of antitumor activity of the mushroom extracts against carcinoma cells lines:

Three carcinoma cell lines were used to test the antitumor activity of the mushroom 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, and the human cervical cancer cells (HeLa) (ATCC® CCL-2™) from an adenocarcinoma.

The HepG2 cell lines were maintained in RPMI 1640 (Life Technologies Inc., Grand Island, NY, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Gemini Bio-Products Inc., West Sacramento, CA. USA), 5 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid - Life Technologies Inc., Grand Island, NY, USA) (pH 7.3), 5 mM sodium pyruvate (Life Technologies Inc., Grand Island, NY, USA) 5 mM non-essential amino acid (Life Technologies Inc., Grand Island, NY, USA) and 5 mM penicillin/streptomycin solution pen/strep (10,000 U/µg/ml; Life Technologies Inc., Grand Island, NY, USA). The HCT 116 and HeLa cell lines were maintained in modified Dulbecco's modified minimum essential medium (DMEM) (Life Technologies Inc., Grand Island, NY. USA) supplemented with 10% (v/v) heat-inactivated FBS, 2 mM glutamine, and 5 mM pen/step. The three carcinoma cell lines were incubated in 75 cm^2 culture corning flask (Fisher Scientific Inc., Loughborough, Leicester, United Kingdom) at 37°C in a water jacketed incubator (Forma Scientific series II, (Thermo Scientific Inc., Waltham, MA, USA). An inverted microscope (Olympus, CKX41; Shinjuku, Tokyo, Japan) was used to view the cell monolayer and confirm the absence of bacterial and fungal contaminants. 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 maintainace 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).

For antitumor assays, cells were suspended in medium at concentration $5x10^4$ cell/well in 200 µl/well of Corning 96-well tissue culture plates (Fisher Scientific Inc., Loughborough, Leicester, United Kingdom), then incubated for 24 hr. Mushroom extracts were then dispensed into 96-well plates in 50 µl volumes to achieve six concentrations for each extract = 50, 25, 12.5, 6.25, 3.125, 1.56 µg/ml, and each concentration was repeated 5 times. Five vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. 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. As follow: The media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red (Life Technologies) then 10 µl 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 plate was then incubated at 37°C and 5% CO2 for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl 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 antitumor activity of the mushroom extracts 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 (Mosmann, 1983; Wu and Wang, 2010; Elaasser et al., 2011).

The half maximal inhibitory concentration (IC_{50}) was determined after plotting the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA).

3. Results:

3.1- The antitumor activities of *F. velutipes* extracts:

The antitumor activity of *F. velutipes* extracts against the three carcinoma cell lines is shown in Table 1. Where the maximal inhibition of cell viability was determined with 50 µg/ml of each extract, and IC50 was determined from a range of concentrations shown in Figures 1-3. All values are mean ± SD.

The water extracts consistently showed the highest antitumor effect whereas ether extracts showed the lowest antitumor effect against the three carcinoma cells. The water extract of lyophilized fruiting bodies showed the highest antitumor effect with maximum inhibitory percentages of 92.9±2.1,

90.9±2.1 and 84.5±1.4 % against Hep G2, HCT 116 and HeLa cells with IC_{50} 7.3±0.7, 6.9±0.5 and 5.2±0.4 µg/ml, respectively. Also, the water extract of fresh fruiting bodies showed high maximum inhibitory percentages of 92.3±1.8, 85.9±1.9 and 78.2±1.9 % against Hep G2, HCT 116 and HeLa cells with IC_{50} 10.6±0.8, 14.3±0.9 and 16.5±0.9 µg/ml, respectively. In comparison, the methanol extract of fresh and lyophilized fruiting bodies showed lower antitumor effect than the water extracts with maximal inhibition ranged between 74 – 35%.

The ethyl acetate extracts and water extract of mycelia and broth showed moderate inhibitory effect against the three tumor cell lines (Fig. 1-3) where the highest observed by ethyl acetate of lyophilized fruiting bodies against Hep G2 cells with IC_{50} 35.9±1.9 µg/ml and the lowest observed by ethyl acetate of broth against Hep G2 cells with IC_{50} 17.7±0.6 µg/ml. On the other hand, ether extract from all *F. velutipes* parts showed the lowest inhibition effect with maximal activity (48-5 %) against most tumor cell lines, where the lowest effect was observed against HCT 116 tumor cells (Fig.1-3).

3.2- The antitumor activities of *G. lucidum* extracts:

G. lucidum extracts in Different solvents exhibited different levels of antitumor against the three cancer cell lines, with lower effects than the *F. velutipes* extracts. The water extracts of lyophilized fruiting bodies showed the highest antitumor effect with maximal inhibition of 87.5±2.1, 87±2.5 and 76.4±2.6 % against Hep G2, HCT 116 and HeLa cells and with IC_{50} 10.5±1.1, 7.9±0.6 and 21.4±2.1 µg/ml, respectively. The water extracts of fresh fruiting bodies those showed maximal inhibition of 80±2.9, 71.8±1.2 and 69.4±2.5 % against Hep G2, HCT 116 and HeLa cells with IC_{50} 8.9±0.9, 17.7±0.9 and 25.7±1.8 µg/ml, respectively.

In comparison, the methanol extract showed less effect than the water extract against the three-carcinoma cells. The methanol extract of both fresh and lyophilized fruiting bodies had approximately the same ranges of maximal activity (79-58 %) and IC_{50} (16-34 µg/ml) against most tumor cell lines. In contrast, the ethyl acetate extract and ether extract of fresh and lyophilized fruiting bodies showed lower effect than water and methanol extracts with IC_{50} ranged between 45-114 µg/ml (Fig.4-6).

The water extracts from mycelia and broth showed moderate effects on the three cell lines. The highest effect was shown by the water extract of mycelia against HCT 116 with maximal activity 73.3±1.9 % and IC_{50} of 26.2±1.6 µg/ml. The methanol, ethyl acetate and ether extracts from mycelia and broth showed lower inhibitory effect against the three tumor cell lines. The lowest inhibitory effect was observed with the ether extract of mycelia against HeLa cells with maximal activity 7.2±0.2 % (Fig.4-6).

3.3- The antitumor activities of *P. eryngii* extracts:

The in vitro antitumor activities of *P. eryngii* showed lower inhibitory effect against the three carcinoma cell lines than both *F. velutipes* and *G. lucidum* extracts (Table 3). The highest inhibitory effect was observed with the water extract of lyophilized fruiting bodies with maximal activity 68.3±1.2, 69.3±1.8 and 72.1±1.9 % against Hep G2, HCT 116 and HeLa cells with IC_{50} 22.1±1.5, 19.1±0.8 and 17.1±0.9 µg/ml, respectively. The water extract of fresh fruiting bodies had maximal inhibition of 59.1±2.2, 60.5±1.9 and 61.7±1.6 % against Hep G2, HCT 116 and HeLa cells with IC_{50} 29.9±2.1, 34.8±1.6 and 33.5±1.6 µg/ml, respectively (Fig. 7-9).

In contrast, the ethyl acetate extract of fresh and lyophilized fruiting bodies had higher inhibitory effect than the methanol and ether extracts but lower than the water extracts with approximately the same ranges of maximal activity (59-32 %) and IC_{50} values of (33-67µg/ml) against the three cell lines.

Methanol and ether extracts of fresh and lyophilized fruiting bodies showed the lowest inhibitory effect against the three tumor cells, with the highest effect against HeLa cells with IC_{50} of 41.2±2.3 µg/ml by ether extract of lyophilized fruiting bodies (Table 3).

Moreover, extracts from broth and mycelia had lower antitumor effect against the three cell line than the fruiting bodies extracts, except the effect of water and methanol extracts of mycelia and water extract of broth against HeLa cells exhibited maximal activity of 61.9±2.5 and 54.9±1.5 and 50.3±1.9 %, respectively (Fig. 7-9). The lowest effect was observed with the ether extract of mycelia against Hep G2 with maximal inhibition12.8±1.8 %.

Table 1. The antitumor activities of *F. velutipes* extracts against the three carcinoma cell lines.

Sample	Solvent	HepG2		HCT 116		HeLa	
		Maximum inhibitory %	IC_{50} (μg/ml)	Maximum inhibitory %	IC_{50} (μg/ml)	Maximum inhibitory %	IC_{50} (μg/ml)
Fresh fruiting bodies	Water	92.3±1.8	10.6±0.8	85.9±1.9	14.3±0.9	78.2±1.9	16.5±0.9
	Methanol	66.3±1.9	32.3±1.6	43.3±1.6	54.5±2.6	64.4±2.1	38.1±2.2
	Ether	32.8±1.9	63.8±2.9	27.9±2.1	69.2±2.9	38.9±3.1	64.3±3.2
	Ethyl Acetate	40.3±2.1	59.3±2.6	19.9±1.4	75.1±3.4	50.9±1.5	50.7±2.5
Lyophilized fruiting bodies	Water	92.9±2.1	7.3±0.7	90.9±2.1	6.9±0.5	84.5±1.4	5.2±0.4
	Methanol	74.1±2.7	30.8±1.7	35.7±1.2	74.7±3.5	71.4±2.5	31.6±2.3
	Ether	48.8±1.5	51.2±2.5	30.1±1.7	79.6±3.7	41.2±2.2	57.9±2.5
	Ethyl Acetate	60.9±1.9	35.9±1.9	55±2.5	40.9±2.1	60±2.4	38.9±2.1
Mycelia	Water	49.1±1.2	51.1±2.2	45.2±2.2	57.6±2.6	42.9±1.8	65.3±2.8
	Methanol	27±1.2	68.8±2.9	33.5±3.1	74.5±3.5	39.6±1.5	70.9±3.5
	Ether	9.1±0.5	448±3.5	16.9±1.8	388±4.8	9.3±0.8	248±3.8
	Ethyl Acetate	17.7±0.6	324±3.6	35.5±2.1	68.5±3.1	41.9±0.9	57.2±2.9
Broth	Water	39.2±1.1	62.2±3.1	36.4±1.4	60.4±3.2	56.3±1.1	45.5±2.5
	Methanol	17.4±2.1	200±2.5	32.2±2.1	65.9±2.9	45±2.1	54.8±2.8
	Ether	10.5±0.9	237±3.9	5.1±0.8	408±5.8	21.7±1.2	195±3.4
	Ethyl Acetate	26.7±0.8	248±3.5	42.4±1.8	58.7±2.8	31±0.9	69.3±2.9

Table 2. The antitumor activities of *G. lucidum* extracts against the three carcinoma cell lines.

Sample	Solvent	HepG2		HCT 116		HeLa	
		Maximum inhibitory %	IC_{50} (μg/ml)	Maximum inhibitory %	IC_{50} (μg/ml)	Maximum inhibitory %	IC_{50} (μg/ml)
Fresh fruiting bodies	Water	80±2.9	8.9±0.9	71.8±1.2	17.7±0.9	69.4±2.5	25.7±1.8
	Methanol	74.6±2.3	17.3±1.2	58.8±2.1	34.3±2.4	62.7±2.7	32.7±1.9
	Ether	26.9±1.9	73.2±3.9	46.7±2.2	51.3±2.6	36.4±2.5	78.8±3.5
	Ethyl Acetate	40.6±2.1	57.6±3.2	45.3±2.0	51.9±2.3	44.4±2.1	56.4±3.1
Lyophilized fruiting bodies	Water	87.5±2.1	10.5±1.1	87±2.5	7.9±0.6	76.4±2.6	21.4±2.1
	Methanol	78.9±2.3	15.9±1.4	73.1±1.9	18.5±0.9	63.1±2.7	31.1±2.1
	Ether	22±1.2	114±3.1	37.8±1.9	68.5±2.6	39.1±2.9	65.2±2.9
	Ethyl Acetate	33.1±1.3	68.6±3.3	51.6±1.9	45.6±1.7	42.7±2.5	58.8±2.4
Mycelia	Water	39.7±2.5	74.6±3.5	35.9±2.2	76.6±2.8	45.9±0.4	44.7±2.5
	Methanol	47.4±2.3	65.6±3.5	46.1±2.1	52.4±2.2	32.3±1.4	72.1±3.4
	Ether	13.2±1.2	272±3.5	21.3±1.1	179.5±2.9	7.2±0.2	336±5.1
	Ethyl Acetate	25.1±2.5	372±7.1	28±1.2	77.4±2.7	17.2±0.9	196±3.2
Broth	Water	36.2±1.4	62.6±3.4	73.3±1.9	26.2±1.6	70.8±2.2	31.2±2.1
	Methanol	32.6±2.1	66.2±3.3	62.1±2.8	34±1.8	30.3±1.5	79.7±3.5
	Ether	10.5±0.9	356±6.1	32.7±2.1	77.3±2.8	24.7±1.2	115±2.2
	Ethyl Acetate	25.2±1.1	352±8.1	44.8±2.1	52.8±2.3	36.4±1.3	70.7±3.4

Table 3. The antitumor activities of *P. eryngii* extracts against the three carcinoma cell lines.

Sample	Solvent	HepG2		HCT 116		HeLa	
		Maximum inhibitory %	IC_{50} (μg/ml)	Maximum inhibitory %	IC_{50} (μg/ml)	Maximum inhibitory %	IC_{50} (μg/ml)
Fresh fruiting bodies	Water	59.1±2.2	29.9±2.1	60.5±1.9	34.8±1.6	61.7±1.6	33.5±1.6
	Methanol	22.4±1.9	244±4.9	30.1±2.9	82.3±3.9	32.1±1.3	69.1±3.3
	Ether	41.1±2.8	76.7±3.8	45.3±2.1	53.1±2.4	53.7±2.4	41.2±2.3
	Ethyl Acetate	32±1.7	67.1±3.7	35.6±1.8	63.2±2.8	53.4±2.5	45.6±2.3
Lyophilized fruiting bodies	Water	68.3±1.2	22.1±1.5	69.3±1.8	19.1±0.8	72.1±1.9	17.1±0.9
	Methanol	24.1±1.9	161±3.9	53.4±1.5	40.7±1.9	38.4±1.4	60.1±3.6
	Ether	42.2±1.2	58.5±2.5	41.1±2.5	57.4±2.3	23.2±2.2	110±3.7
	Ethyl Acetate	33.9±1.5	63.9±3.5	35.6±2.9	63.1±2.8	59.6±2.2	33.7±2.3
Mycelia	Water	29.8±2.6	70.1±3.6	34±2.2	69.1±3.2	61.9±2.5	34.2±2.3
	Methanol	14.6±1.6	204±4.6	17.1±1.1	404±4.6	54.9±1.5	41.8±2.5
	Ether	32.3±1.1	87.3±4.2	32.3±1.8	87.3±3.8	17.2±1.9	113±3.7
	Ethyl Acetate	12.8±1.8	208±4.6	42.3±2.3	55.7±3.3	22.3±1.8	103±3.8
Broth	Water	28.1±1.4	368±5.6	38.7±1.5	63.6±3.5	39.4±1.5	60.1±2.8
	Methanol	25.2±1.3	398±6.8	27.6±1.4	80.2±3.9	50.3±1.9	47.5±2.7
	Ether	16.9±1.5	493±7.5	19.8±1.5	493±7.5	20.9±1.1	120±3.9
	Ethyl Acetate	21.4±1.4	368±4.9	25.1±1.5	91.3±4.1	24.9±1.1	93.6±4.5

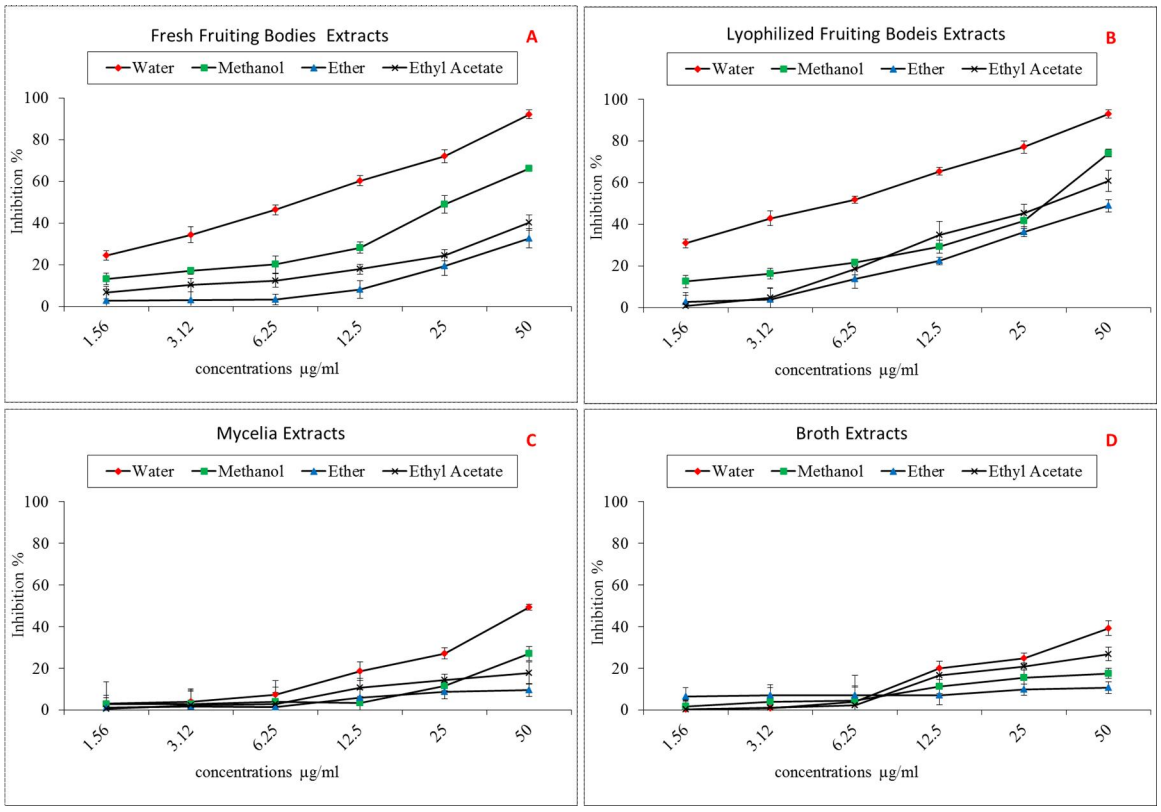

Fig. 1. The antitumor activities of *F. velutipes* extracts against Hep G2 cell line.

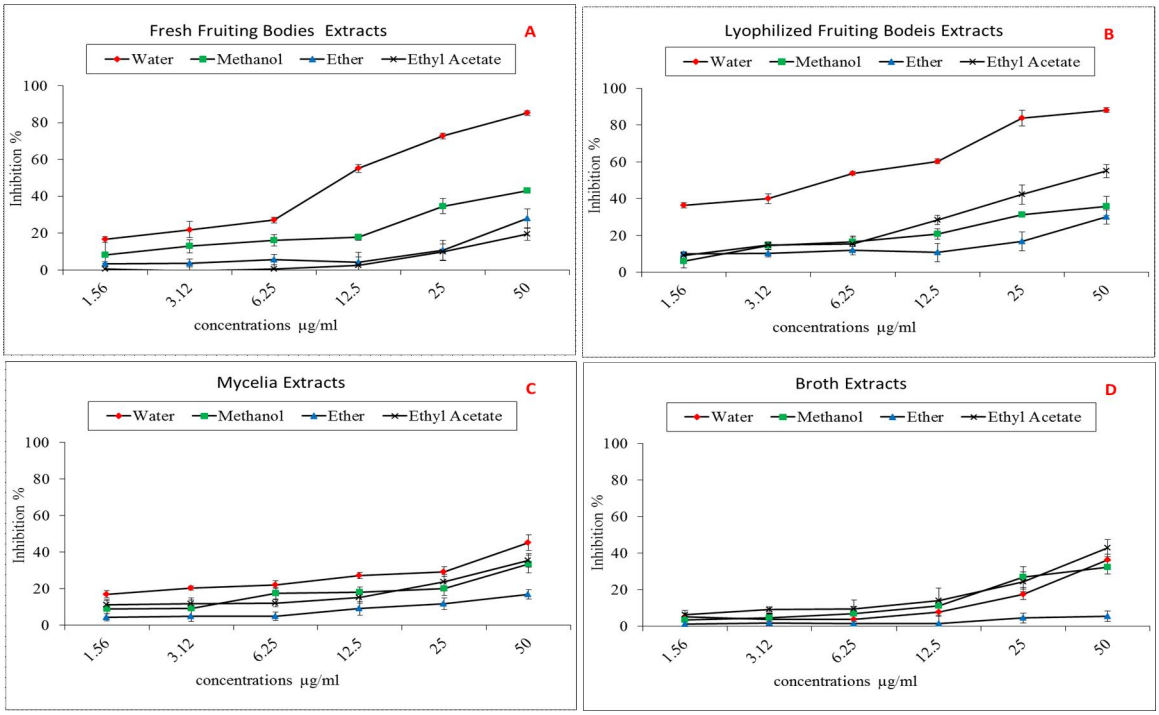

Fig. 2. The antitumor activities of *F. velutipes* extracts against HCT 116 cell line.

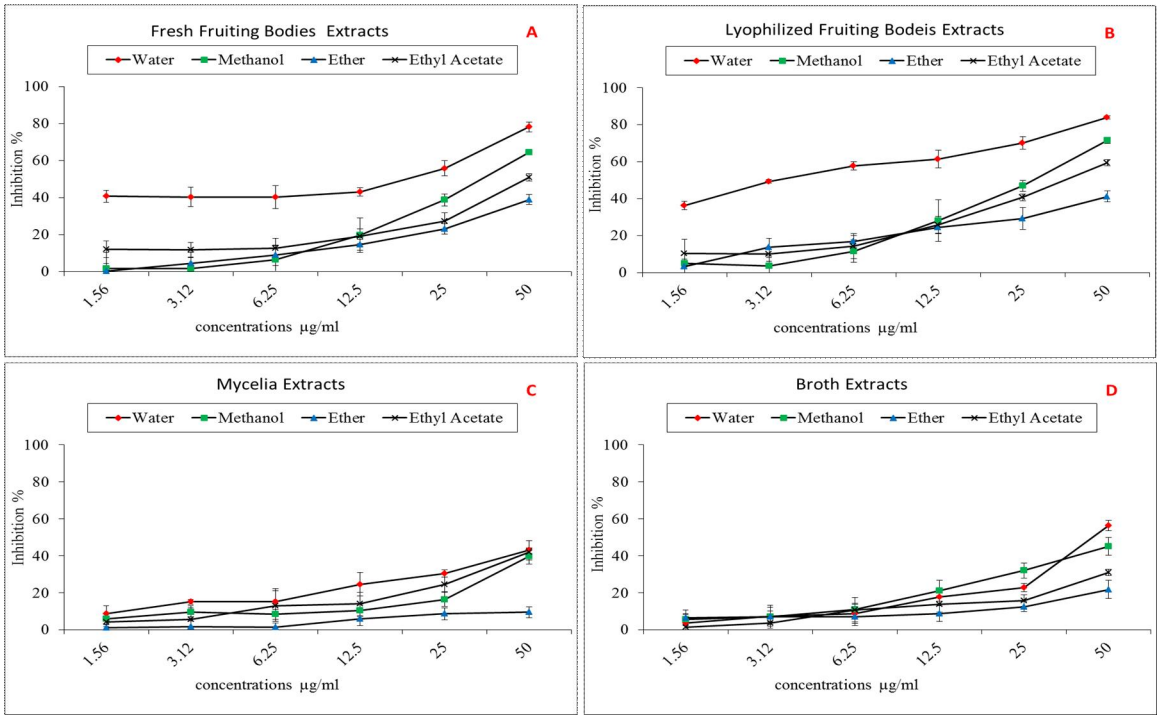

Fig. 3. The antitumor activities of *F. velutipes* extracts against HeLa cell line.

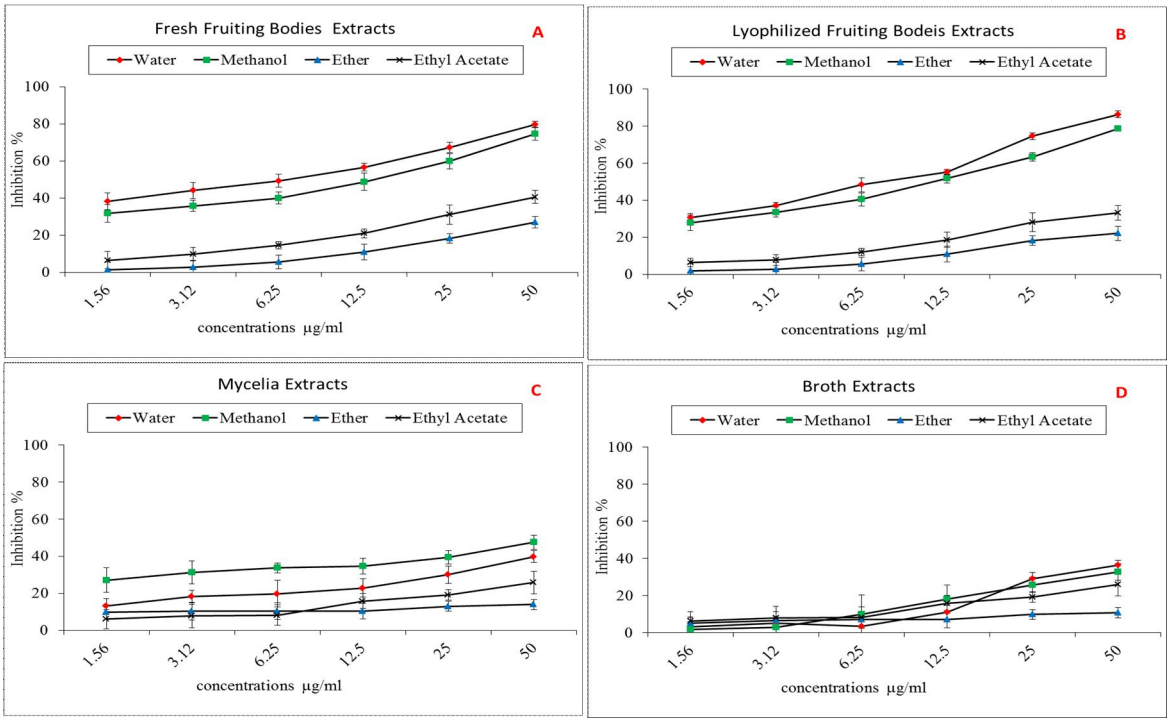

Fig. 4. The antitumor activities of *G. lucidum* extracts against Hep G2 cell line.

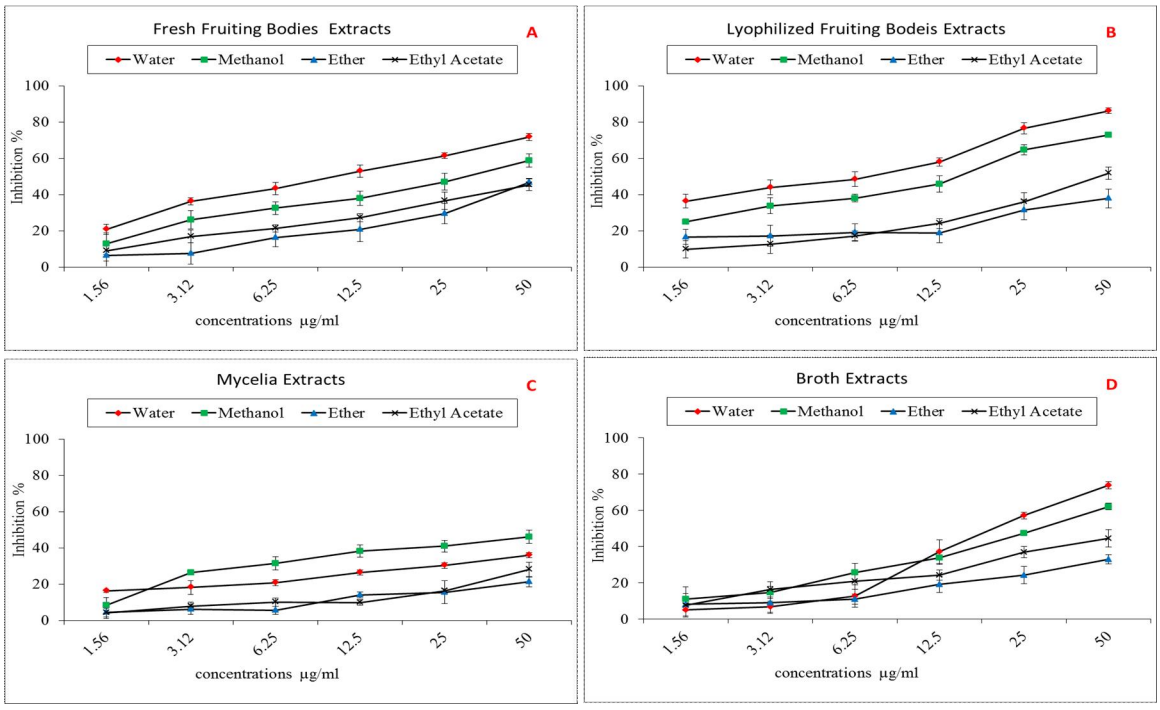

Fig. 5. The antitumor activities of *G. lucidum* extracts against HCT 116 cell line.

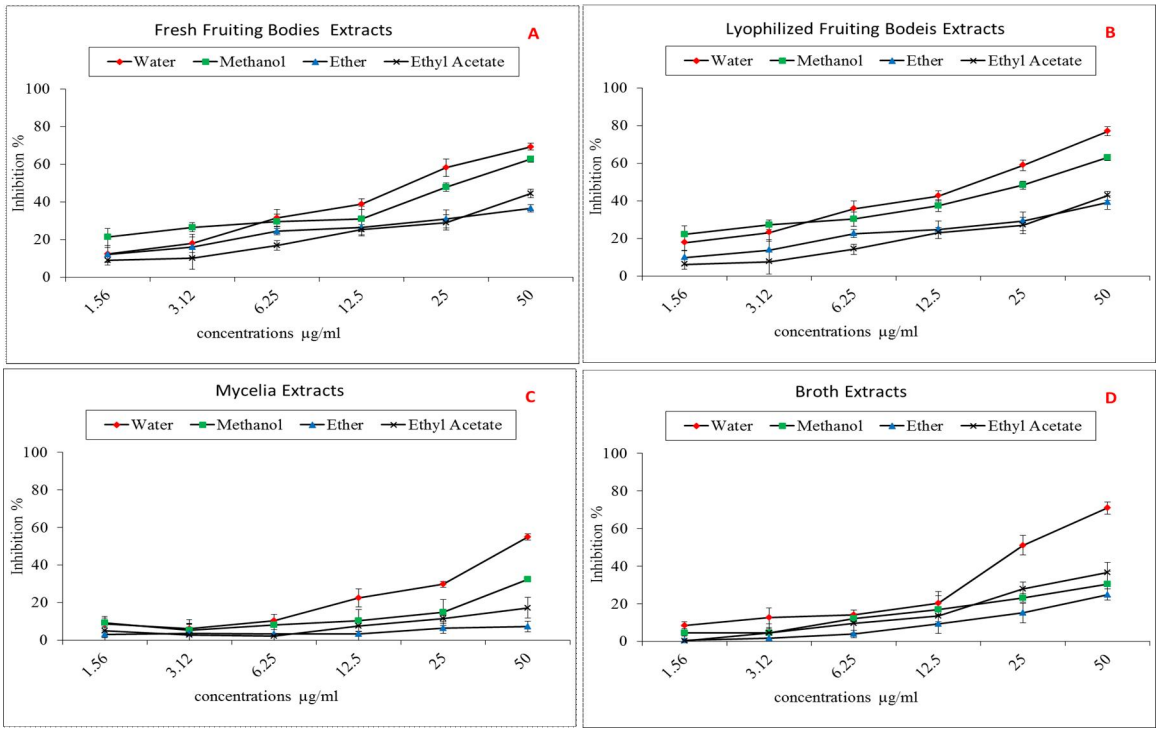

Fig. 6. The antitumor activities of *G. lucidum* extracts against HeLa cell line.

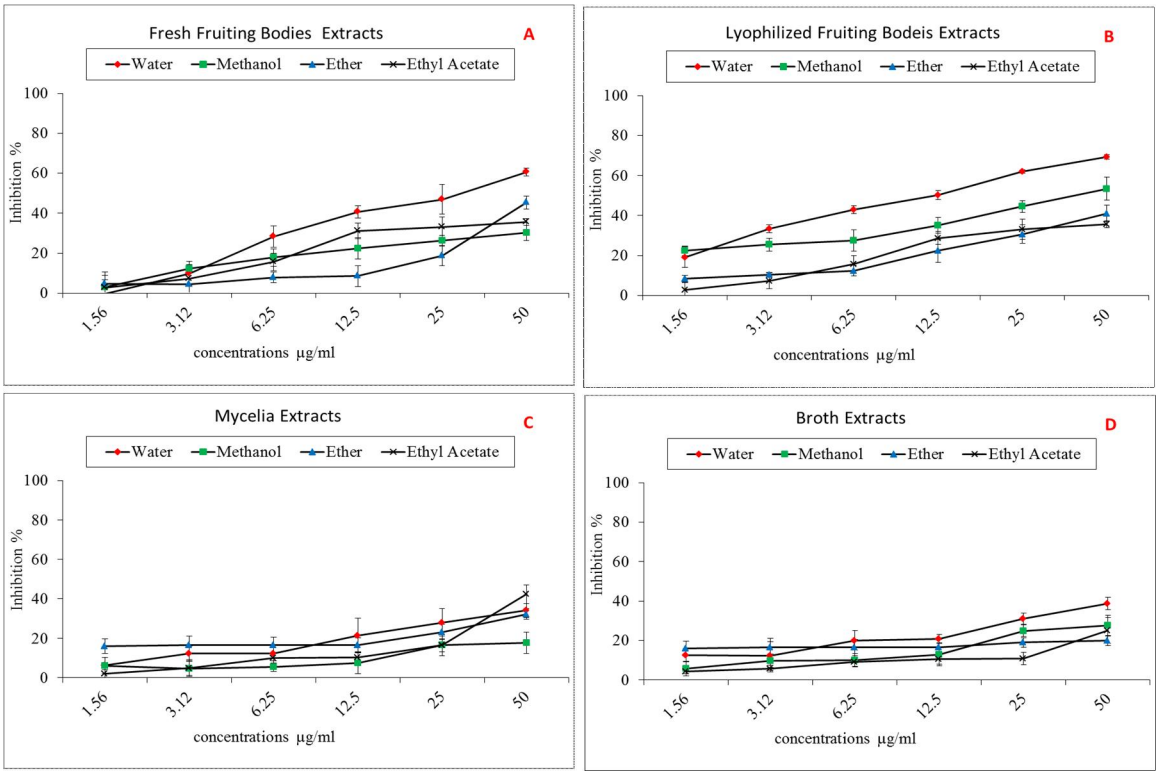

Fig. 7. The cytotoxicity activities of *P. eryngii* extracts against Hep G2 cell line.

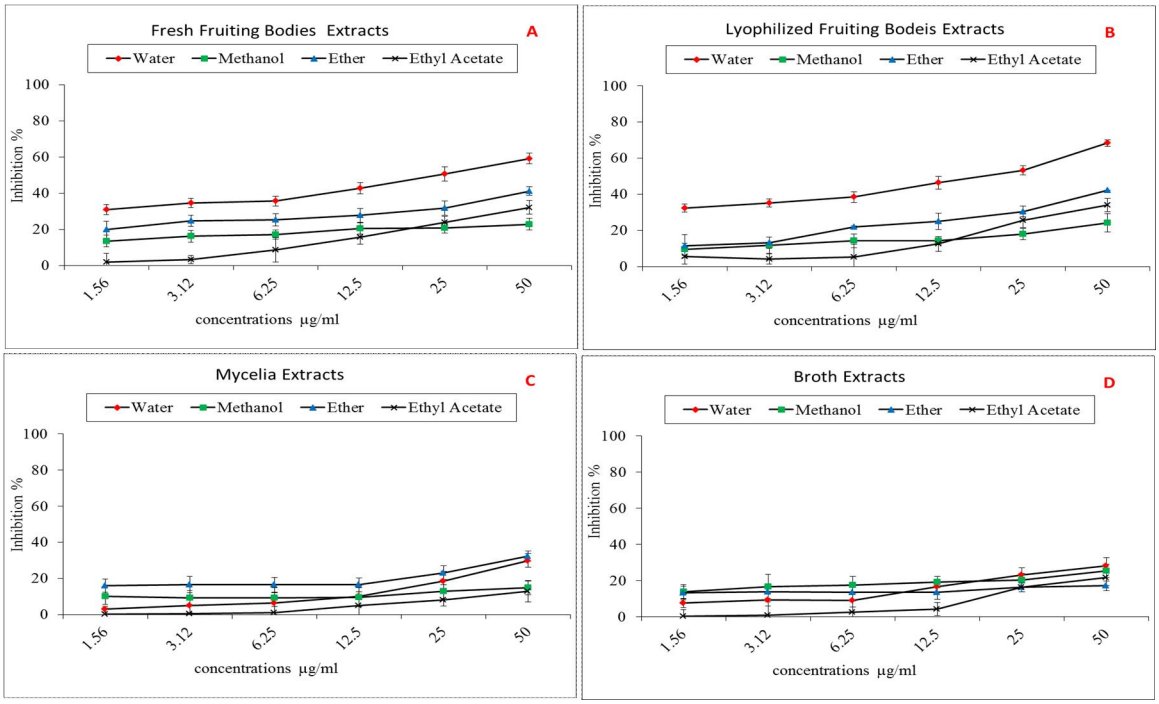

Fig. 8. The antitumor activities of *P. eryngii* extracts against HCT 116 cell line.

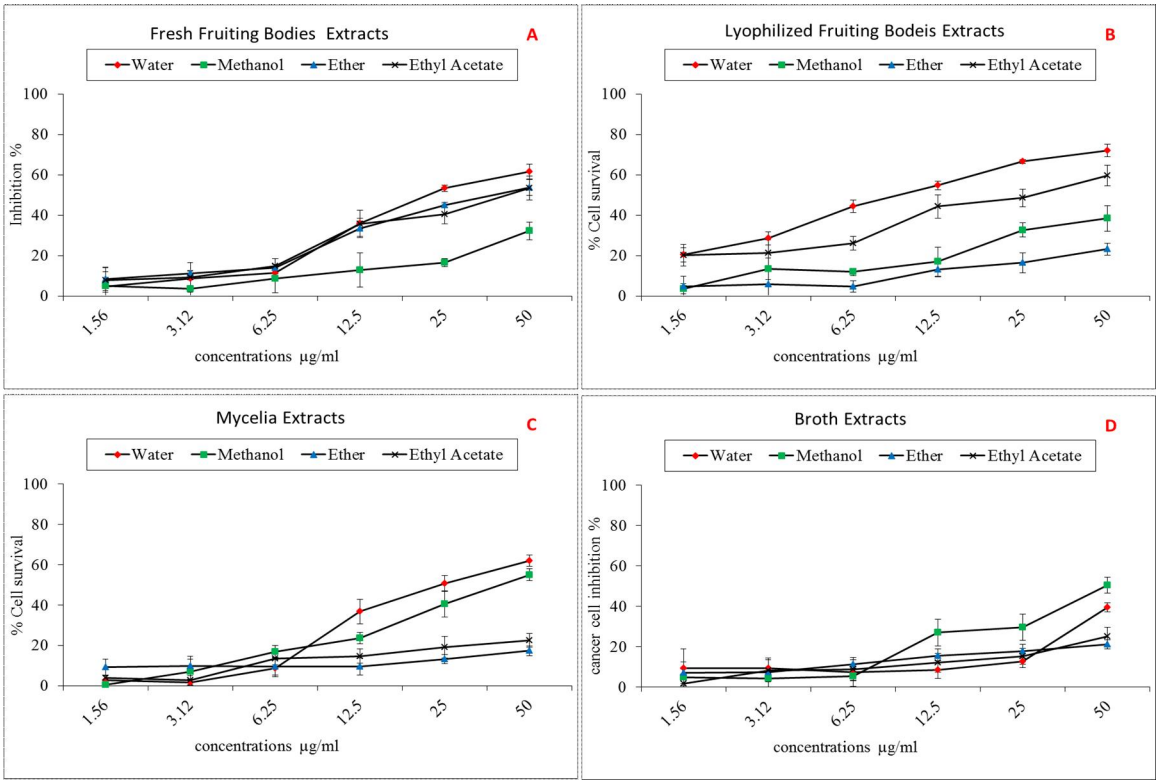

Fig. 9. The antitumor activities of *P. eryngii* extracts against HeLa cell line.

4. Discussion:

The treatment options of cancer including surgery, chemotherapy, radiation therapy, and palliative care often are expensive and have side effects. For example, most chemotherapeutic agents for the treatment of cancer destroy tumors and stop cancer progress but also damage healthy cells and tissues. Due to these side effects, researchers search for new antitumor substances from various natural sources, especially fungi (Cui et al., 2011; Rajendran et al., 2013; Evidente et al., 2014). More than 140 fungal metabolites have shown confirmed activity in tumor cell line bioassays (Wang et al., 2011). Although *F. velutipes, G. lucidum* and *P. eryngii,* are also fungi, their antitumor activities have not been fully exploited.

Our results demonstrated that mushroom extracts can be a good source for antitumor substances, and these results are consistent with reports indicating that mushrooms extracts had antitumor effects. For examples, low-molecular weight mushroom substances have been studied, and reported to interact with particular intracellular signaling pathways related to processes such as inflammation, cell differentiation and survival, apoptosis, angiogenesis, tumor progression and metastasis (Petrova, 2012). Many polysaccharide or polysaccharide protein complexes isolated from mushrooms exhibit antitumor activity (Wasser, 2002; Ma et al., 2013). Lentinan, a polysaccharide isolated from water extract of *L. edodes* has strong antitumor activities against stomach cancer (Ng and Yap, 2002; Kim et al., 2007; Ina et al., 2013). Also, Finimundy et al. (2013) reported high inhibitory activities against both HeLa and Hep G2 cell lines by water extracts of *L. edodes*.

Our results clearly indicated that the extracts from lyophilized fruiting bodies were the most potent extracts with inhibitory effects against the tumor cells followed by the extracts from fresh fruiting bodies. In contrast, the extracts from mycelia and broth had the least antitumor activity against the three carcinoma cell lines. Also, in general the extracts by water and methanol had a stronger inhibitory effect than the extracts by ether and ethyl acetate.

In our results, water extracts of *F. velutipes* fruiting bodies showed the highest inhibition effect against the three tumor cells with IC_{50} ranging between 5-16 µg/ml. This result is similar to reports observed by Ikekawa et al. (1983) who isolated an antitumor polysaccharide EA501 from *F. velutipes* composed of D-glucose 42.3%, D-galactose 17.3%, D-mannose 12.2%, D-xylose 6.7% and L-arabinose 14.7%. A similar polysaccharide has been isolated from *F. velutipes* with antitumor activity (Leung *et al.,* 1997). Also, a protein-bound polysaccharideisolated from *F. velutipes* can prolong the lifespan of mice challenged with leukemic cells (Otagiri*et* et al., 1983).

Several ribosome inactivating proteins have been purified from *F. velutipes,* including flammulin (40 kDa) (Wang and Ng, 2000), velutin (13.8 kDa) (Wang and Ng, 2001), flammin (30 kDa) and velin (19 kDa) (Ng and Wang, 2004).

In our study the methanol and water extracts of *G. lucidum* fruiting bodies showed highly inhibitory activities against the three tumor cells with IC_{50} ranging between 7.9±0.6 and 32.7±1.9 µg/ml. Other studies also show that *G. lucidum* extracts possess anti-proliferative effects on many tumor cell lines in vitro (Suarez-Arroyo et al., 2013), such as anti-proliferative effects against a colorectal prostate cancer cell line (Berovic et al., 2003), lung cancer cell line (Jiang et al., 2005), acute myelogenous leukemia cancer cell line (Cheng et al., 2007), breast cancer cell line (Thyagarajan et al., 2006), colorectal cancer cell line (Xie et al., 2006), and bladder cancer cell line (Paterson, 2006). The water extract and the polysaccharides fraction of *G. lucidum* exhibited significant antitumor effect in several tumor-bearing animals, mainly through its immunoenhancing activity (Wang and Wang, 2006). Studies also showed that the alcohol extract or the triterpene fraction of *G. lucidum* possessed antitumor effect, which seemed to be related to the antitumor activity against tumor cells directly (Lin and Zhang, 2004).

In our study, the water extracts of *P. eryngii* fruiting bodies showed the highest inhibitory activities by *P. eryngii* extracts but lower than water extracts of *F. velutipes* and *G. lucidum* fruiting bodies against the three tumor cells with IC_{50} ranging between 17.1±0.9 and 34.8±1.6 µg/ml. There are also many reports of antitumor effects of water-soluble polysaccharide isolated from *P. eryngii* (Zhang et al., 2013; Kawai et al., 2014; Fontana et al., 2014). In addition, Ma et al. (2014) isolated three water-soluble polysaccharides named PEPE-1, PEPE-2 and PEPE-3 from *P. eryngii* and mainly composed of glucose, sulfate and uronic acid content with antitumor activities against HepG-2. Also, Yang et al. (2013) reported significant inhibition of the tumor growth of mice bearing renal cancer with water-soluble polysaccharide isolated from *P. eryngii* at the doses of 50, 100 and 200mg/kg.

Cancer is a leading cause of death worldwide resulting in 8.2 million or 14.6% of all human deaths, with financial costs estimated at $1.16 trillion US dollars per year (WHO, 2014). Moreover, new antitumor agents that are available on the market are very limited and with unwanted side effects (Chang et al., 2010). The discovery of new antitumor compounds is therefore becoming more important. The facts that edible mushrooms are non-toxic and

contain various compounds beneficial to human health; encouraged us to study the mushrooms activities as a sources of antitumor drugs. Our finding that the water extracts from the three mushrooms *F. velutipes, G. lucidum* and *P. eryngii* contain antitumor compounds that are effective against the three tumor cell lines. In addition, these active mushroom extracts can be used in combination with traditional chemotherapy or might be considered alternative sources for adjuvant cancer therapy, as they have no unfavorable effects and they activate the cells of the immune system (Barros et al., 2007; Jedinak and Sliva, 2008; Patel and Goyal, 2012).

More studies will be needed to isolate and identify the active compounds and determine the antitumor effect of these active compounds, as well as the effects on normal healthy cells. We believe that it is worthwhile to exploit the potential of these antitumor compounds in treating the cancer diseases.

Author contributions:
Younis A, Stewart J and Elaasser M contributed equally to this work; Wu F, El Shikh H and Hassan F designed the research; Younis A and Stewart J performed the research; Younis A and Stewart J analyzed the data; Younis A and Elaasser M wrote the paper.

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