Anti-Cancer Potential of the Polyphenolic- Polysaccharides –Protein Complex Extracted from Edible Mushroom

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Abstract

Background: Cancer, as one of the most life-threatening diseases, has attracted the attention of researchers. Recently, antitumor drugs and other biologically active compounds have been discovered in many mushroom species. The aim of this study is to the anti-cancer activity of polysaccharide-protein complexes.

Methods: This study applies new method for the extraction, isolation and purification of polysaccharide-protein complexes and identifies the anticancer effect. MTT assay counts the number of live cells by measuring mitochondrial activity. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), a yellow substrate, is converted by active mitochondria into the purple product formazan.

Results: The results of the analysis showed that the highest amount of mushroom powder was protein (37, 9%), carbohydrates (23, 82%), as the results of the mushroom extract used in this study showed. LC/HPLC showed that the presence of high content of protein 53 that, play potent role in anti-cancer activity. The effect of polysaccharide-protein complexes on normal cells was low (IC50 ٧٤,٦٥ µg/mL), while, the effect on liver cancer cells was moderate (IC_{50} ٥٦,٢ µg/mL), and the effect on colon cancer cells was high (IC_{50} ± 23.1µg/mL).

Conclusion: Mushroom powder and extract are readily available as a source of polyphenolic-polysaccharide-protein complex with key antioxidants which are functional food components as they affect physiological and biochemical processes leading to better health and improved health status in cancer patients.

Keywords: protein and polysaccharide; health and nutrition; Protein 53; Chemical Composition.

الإمكانات المضادة للسرطان لمتراكب متعدد الفينول متعدد السكاريد البروتيني المستخلص من المشروم الصالح للأكل.

ملخص البحث: لقد جذب السرطان، باعتباره أحد أكثر الأمراض التي تهدد الحياة، انتباه الباحثين. وفي الآونة الأخيرة، تم اكتشاف أدوية مضادة للأورام ومركبات نشطة بيولوجيًا أخرى في العديد من أنواع الفطر. يحتوي الفطر على العديد من أنواع المركبات الكيميائية الأساسية بما في ذلك مركب البوليفينول – متعدد السكاريد – البروتين. والهدف من هذه الدراسة هو تحديد التأثير المضاد للسرطان لمركب البوليفينول – متعدد السكاريد – البروتين، وتطبيق طرق جديدة لاستخراج وعزل وتنقية مجمعات البوليفينول – البروتين.

الطريقة:

تطبق هذه الدراسة طريقة جديدة لاستخلاص وعزل وتنقية معقدات البروتين متعدد السكاريد وتحديد التأثير المضاد للسرطان. يقوم اختبار MTT بحساب عدد الخلايا الحية من خلال قياس نشاط الميتوكوندريا. يتم تحويل بروميد ٣-(٤,٥--dimethylthiazol-2-yl)-٢,٥-diphenyltetrazolium (MTT)، وهو ركيزة صفراء، بواسطة الميتوكوندريا النشطة إلى منتج أرجواني هو الفورمازان.

النتائج:

أظهرت نتائج التحليل أن أعلى كمية من مسحوق الفطر كانت البروتين (٣٧,٩)، والكربوهيدرات (٢٣,٨٢)، كما أظهرت نتائج مستخلص الفطر المستخدم في هذه الدراسة، وكان تأثير معقدات السكاريد البروتينية على الخلايا الطبيعية منخفضًا (IC_{50} ±74.65 µg)، بينما كان التأثير على خلايا سرطان الكبد متوسطًا (IC_{50} ±56.2 µg)، وكان التأثير على خلايا سرطان القولون مرتفعًا (IC_{50} ± 23.1µg).

الخلاصة:

أن مسحوق ومستخلص المشروم الصالح للأكل يحتوى على متراكب متعدد الفينول متعدد السكاريد البروتيني ٥٤٠ تعمل كنشاط مضاد للسرطان التى ظهرت فاعليتها وتأثيرها على سرطان القولون بنسبة عالية وسرطان الكبد بنسبة متوسطة والخلايا الطبيعية بنسبة بسيطة.

الكلمات المفتاحية: البروتين والسكريات المتعددة؛ الصحة والتغذية؛ البروتين ٥٣؛ التركيب الكيميائي.

1. Introduction:

Herbal supplements are used in conjunction with most cancer treatments. Medicinal herbs have a positive impact on cancer. Herbal treatments are used in a number of therapies to enhance quality of life. Natural compounds originating from plants, such as alkaloids, terpenes, and flavonoids, have drawn a lot of attention lately because of their many pharmacological characteristics, which include cytotoxic and cancer-chemo preventive activities **(Babu et al., 2002)**. With the discovery and development of vinca alkaloids such as vincristine and vinblastine, as well as the isolation of the deadly podophyllotoxines, the search for anti-cancer medicines derived from plants began in the 1950s. Natural remedies derived from therapeutic plants have been essential in the treatment of cancer. There are fourteen types of natural products or their derivatives. **(Butlet, 2004 &Shoeb, 2006**).

The potential for holistic treatment, including immune system stimulation, has made complementary and alternative medicine, a viable alternative to these therapies. Algae and mushrooms are among the many plant-derived that are utilized extensively throughout the world as immunoceuticals and biological response modifiers (BRMs). **(Kidd. 2000 &Ferlay et al., 2008)**. One of the natural resources provided by nature to help us maintain a healthy lifestyle and live a better life is the mushroom. Thus, mushrooms have been utilized for thousands of years as both food and medicinal. Additionally, various medicinal fungi with anti-cancer potential are shown in the data in Figure 1. **(Kumar *et al.*, 2021).**

Fig. (1): Some medicinal mushrooms with anti-cancer potential

The polysaccharides found in mushrooms (Agaricus arvensis; family: Agaricaceae) have garnered a lot of attention lately because of their anti-cancer and immunomodulatory qualities. **(Damini et al., 2018&Borchers *et al.*, 2008).**

Every year, a large number of toxic and edible mushroom species are grown. Only 100 of the world's 1700 types of mushrooms are edible. **(Kumar *et al.*, 2021).** Beta-glucan and chitin are two significant compounds present in mushroom cell walls. They contain beta-glucans, which are crucial for health and the treatment of numerous ailments. Other significant components are also present in mushrooms in addition to these compounds. These include polysaccharides, agartin, ergosterol, selenium, polyphenols, and protein terpenoids. polysaccharide-protein complexes. These drugs are typically considered biological response modifiers (BRMs) in addition to their therapeutic characteristics. **(Shiu-Nanc *et al.*, 2014).** A wide range of materials, including plants, microbes, algae, and animals, contain polysaccharides, which are carbohydrate molecules made up of long chains of monosaccharides joined by glycosidic bonds. **(Devi N *et al.*, 2017).**

Peptide bonds form a chain of polymers of amino acids that make up proteins. Proteins are the most beneficial macromolecules found in living things since they are essential to every biological process. In addition to being catalysts, they also produce movement, carry and store oxygen, send nerve signals, offer immunological defence and mechanical support, and regulate growth. Linear polymers consisting of up to 20 distinct types of L-α-amino acids make up the majority of proteins. The building blocks of all protein amino acids are the same. The primary, secondary, tertiary, and quaternary levels of complexity are typically used to define protein structure.Polysaccharide shape, content, and size all affect their action. **(Zhang *et al.*, 2007).** While chitin and cellulose are also polysaccharides found in fungal cell walls, the most significant of these carbohydrates are glucose or glucose polymers, which form a variety of glycosidic bonds, including glucan or heteroglycan, which also exhibit the polysaccharides in the fungus's primary cell wall and some biological activities of beta-glucan. **(Ooi *et al.*, 2000).**

Carbohydrates found in the cell walls of many microorganisms, such as fungi, yeast, algae, bacteria, lichen, and plants, are beta-glucans, which are homopolymers of D-glucose and are thought to be the most biologically active polysaccharides. They have antitumor, anti-inflammatory, and immunomodulatory properties. As a result, they are utilised in immuno-oncology therapeutic settings in numerous nations worldwide. **(Laura B *et al.*, 2011).** Nutritional formulations made from medicinal mushrooms may be natural medications with minimal adverse effects, according to clinical research. Mushroom polysaccharides are novel substances that have the potential to improve immune responses. The dosage, concentration, purification techniques, and length of therapy are the only factors that affect a drug's effectiveness when it comes to any natural resource. Several kinds of mushrooms have been found to have therapeutic qualities.

2. Materials and Methods

2.1 Materials

2.1.1 Plant material

In Zagazig City, Governorate, Sharkia, Egypt, edible mushrooms (*Agaricus arvensis*) were purchased from the local market. They were then dried and processed into a fine powder. The Central Laboratory for Breeding, Food, and Feed, Faculty of Technology, Zagazig University, Egypt, is where the plant material was documented. A voucher sample was deposited (voucher number 2024/5/151).

2.2 Methods

2.2.1 Preparation of mushroom powder and extracts:

In order to obtain the extract, 50 g of mushroom powder was soaked in 500 ml of 96% ethanol. The mushrooms were also allowed to dry for four days at room temperature before being ground into a powder in a blender and stored in tightly sealed glass bottles in a dry location until they were needed **(Russo, 2001)**, who stated that herbs should be kept in a dry and dark place to prevent oxidation of their contents.

2.2.2 Extraction of Polyphenolic-Protein-Polysaccharide Complexes:

Two litres of ethanol were used to suspend 200 grammes of dry plant material after it had been cleansed. The ethanol component was then eliminated using filtration. The new ethanol portion was used to repeat the process. To obtain a clear supernatant, the plant residue was centrifuged at 8000 rpm for 15 minutes at room temperature after being suspended in 2 l of 0.1 M NaOH and refluxed for 3 hours at 80 °C. After neutralising the alkaline fraction with 1 M HCl, 30% of the dried plant material was concentrated on a rotary evaporator at lowered pressure. One litre of water and one litre of diethyl ether were used to dissolve the extract at 34 °C. The procedure was repeated using the new diethyl ether component. One litre of chloroform was then added to the combined water extract. The mixture was left for six hours. The new diethyl ether component was used to repeat the process. The combined water extract was then mixed with one litre of chloroform. The mixture was refluxed for six hours at 61 °C. After repeating the chloroform extraction procedure, a 3:1 ethanol and chloroform combination was used to reflux the water-soluble fraction twice for six hours at 70 °C. Following a multi-step extraction procedure utilising organic solvents, the water-soluble fraction was dried off using a rotary evaporator operating at lower pressure. It was agitated for 24 hours at room temperature, then suspended in 500 milliliters of methanol and filtered away. This surgery was performed five times. The precipitate was dialysed against water for six days after drying at room temperature and dissolving in distilled water. **(Wu DT *et al.*, 2020).**

2.2.3 Chemical components of mushroom:

Crude fiber, moisture content, ash, fat and protein were determined. Measurements were based on SI and/or NIST. Environmental conditions during testing (temperature: 22˚C, humidity: 21%).

2.2.4 Chemical analysis

2.2.4.1 LC/HPLC analysis of protein compounds

The protein column is Poroshell 300SB-C18, which is 2.1 x 75 mm and has a thickness of 5 µm. The mobile phase gradient is 20–100% B in 5.5 minutes. B: 0.1% FA or TFA plus ACN A: water plus 0.1% TFA or FA

Flow Rate: 500 µL/min 60°C is the temperature. One microlitre injector BSA at 10 pmol as an example Ionisation by electrospray: positive ion Vcap: 6000V Drying gas: 12L/min at 350°C 45 psi nebuliser The step size for the 600–2500 amu scan is 0.15 amu. Maximum width: 0.06 minutes.

2.2.4.2 HPLC analysis of phenolic compounds

The polyphenolic HPLC analysis was performed using an Agilent 1260 series. For the separation, a Zorbax Eclipse plus C8 column (4.6 mm x 250 mm i.d., 5 µm) was utilised. At a flow rate of 0.9 ml/min, the mobile phase was made up of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). The mobile phase was coded in a linear gradient in the manner described below: 66% A for 11–18 minutes, 82% A for 18–22 minutes, 82% A for 22–24 minutes, 75% A for 1–11 minutes, and 82% A for 0–1 minutes. We noticed the multi-wavelength detector at 280 nm. An injection volume of 5 µl was used for each sample solution. 40 °C was maintained as the column's temperature.

2.2.5 Biological activities

2.2.5.1 Antioxidant activity

Different plant leaf extracts were tested for their ability to scavenge free radicals using 1, 1-diphenyl-2-picryl hydrazyl (DPPH). To put it briefly, a 0.1 mM DPPH solution in ethanol was made. Three millilitres of various extracts in ethanol at varying concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml) were mixed with one millilitre of this solution. Only extracts that are soluble in ethanol are employed here, and the dilution procedure was used to prepare them in different concentrations. After giving the mixture a good shake, it was let to stand for half an hour at room temperature. At 517 nm, absorbance was then measured. using a UV-VIS Milton Roy spectrophotometer. The experiment was conducted in triplicate using ascorbic acid as the reference standard component.16 The sample's IC 50 value, The log dosage inhibition curve was used to determine the sample concentration needed to inhibit 50% of the DPPH free radical. Higher free radical activity was indicated by the reaction mixture's lower absorbance. **(Gonzalez et al., 2016).**

2.2.6 Cytotoxic activity

2.2.6.1 Determination of sample cytotoxicity on cells (MTT protocol)

To create a full monolayer sheet, 96-well tissue culture plates were injected with 1 X 105 cells/ml (100 ul/well) and cultured for 24 hours at 37°C. Following the formation of a confluent sheet of cells, the growth medium was decanted from 96-well microtiter plates and the cell monolayer was twice washed with wash media. The tested sample was diluted twice in RPMI medium (maintenance medium) containing 2%

serum. Three wells served as controls, receiving only maintenance medium, while 0.1 ml of each dilution was examined in separate wells. Plate was incubated at 37°C and inspected. Physical indicators of toxicity, such as rounding, shrinkage, cell granulation, or partial or whole loss of the monolayer, were examined in the cells. A 5 mg/ml MTT solution in PBS was made (BIO BASIC CANADA INC.). Each well received a 20µl addition of MTT solution. To fully incorporate the MTT into the medium, place it on a shaking table and spin it at 150 rpm for five minutes. Allow the MTT to be metabolised by incubating it for four hours at 37°C with 5% CO_2. Get rid of the media. (If required, dry the plate using paper towels to get rid of any residue.Formazan, an MTT metabolic product, should be reconstituted in 200 µl of DMSO. To fully incorporate the formazan into the solvent, place it on a shaking table and spin it at 150 rpm for five minutes. At 560 nm, read the optical density; at 620 nm, subtract the background. There should be a direct correlation between optical density and cell quantity. **(Alley *et al.*, 1988, Slater et al., 1963 and Van de loosdrecht *et al.*, 1994).**

2.2.6.2 Morphological assay

Large-scale morphological alterations that occur at the cell surface or in the cytoskeleton can be connected to cell survival. Damage is indicated by large volume reductions caused by changes in permeability to sodium or potassium or by losses of intracellular ions and proteins. Necrotic cells exhibit chromatin flocculation, nuclear swelling, and a deficiency of nuclear basophils. Apoptosis is indicated by nuclear condensation, nuclear fragmentation, and cell shrinkage. **(Alley *et al.*, 1988), (Slater et al., 1963)**

3. Results and Discussion

3.1 Chemical composition

Table 1 displays all of the findings from the chemical composition study. A summary of the dried mushroom powder's results from this study indicated that it is a nutritious diet that is high in protein (37.9%) and carbs (23.8%).

Table 1: Chemical composition of Mushroom powder (100g D/W)

Parameters	Test result	The method used in measurement
Protein %	37.9±1.44	ES:5465-1/2006
Fats %	7.2±0.20	EN26.2L54/37
Fibers %	11.17±0.83	EN26.2L54/40
Moisture %	10.77±0.19	ES : 5462/2006
Ash %	9.44±0.09	ES : 5464/2006

3.2 Protein-Polysaccharide Complexes

3.2.1 Total Protein compounds

By using a series of aqueous extraction techniques that create foaming with water, protein-polysaccharide natural fractions with different protein and polysaccharide contents were produced.

Generally speaking, additional stabilising agents—polysaccharides being the most popular—are required for proteins to form stable foams. The food industry usually uses polysaccharides because of their textural qualities as thickening and gelling agents. Their interactions with other molecules have been overlooked in most studies on their characteristics as proteins. However, interactions between polysaccharides and proteins are common, and mixtures of polysaccharides usually have synergistic effects. For instance, when used independently as thickening agents in food, guar and xanthan mixtures have strong gelling properties. The theoretical understanding of the stability of protein–polysaccharide mixtures in water has drawn increased attention from the food sector throughout the last ten years. (**Narchi *et al.*, 2009)**

According to the HPLC mechanism (Fig. 2), molecules are sorted by size according to how well they can pass through the column support's pores. To increase the separation range, many columns with varying pore sizes might be assembled. The packing's pore size determines which molecules split in the linear range. This clarified that HPLC is the best method for examining the protein mixture, as seen by the high concentration of protein 540 found in (Fig 3). The most effective cytotoxic agent for cancer cells was found to be protein 540. **(Elhusseiny *et al.*, 2021).**

The transcription factor p53 is referred to as the "guardian of the genome" due to its vital role in maintaining the integrity of the genome. About half of all human cancers, including those of the breast, colon, lung, liver, prostate, bladder, and skin, have a mutation in the TP53 gene. The TP53 gene on human chromosome 17 halts the cell cycle when DNA damage occurs. A mutation in the p53 protein causes the cell cycle to be unchecked and the damaged DNA to be reproduced, which leads to unchecked cell division and cancerous tumours. Typically, phenotypes linked to tumor-associated p53 mutations differ from those resulting from the loss of the tumor-suppressive function of wild-type p53 protein. Due to their oncogenic properties, many of these mutant p53 proteins alter the capacity of cancer cells to divide, evade apoptosis, invade, and spread. This protein is a great choice for cancer treatment because p53 deficiency is so prevalent in human malignancies. **(Marei *et al.*, 2021).**

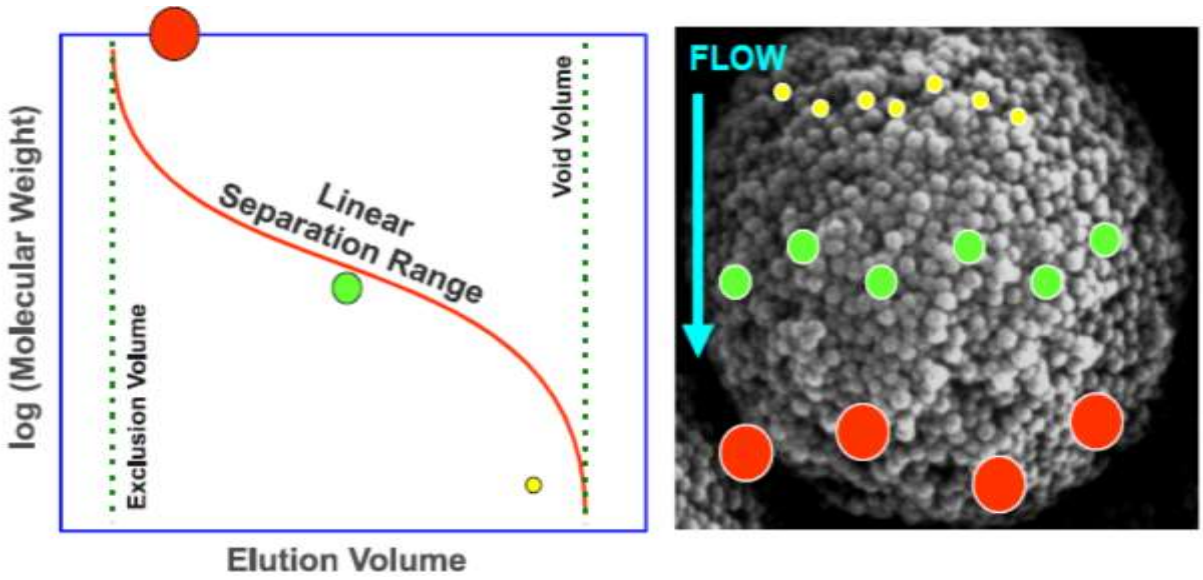


Fig. (2): HPLC Mechanism of the mushroom proteins

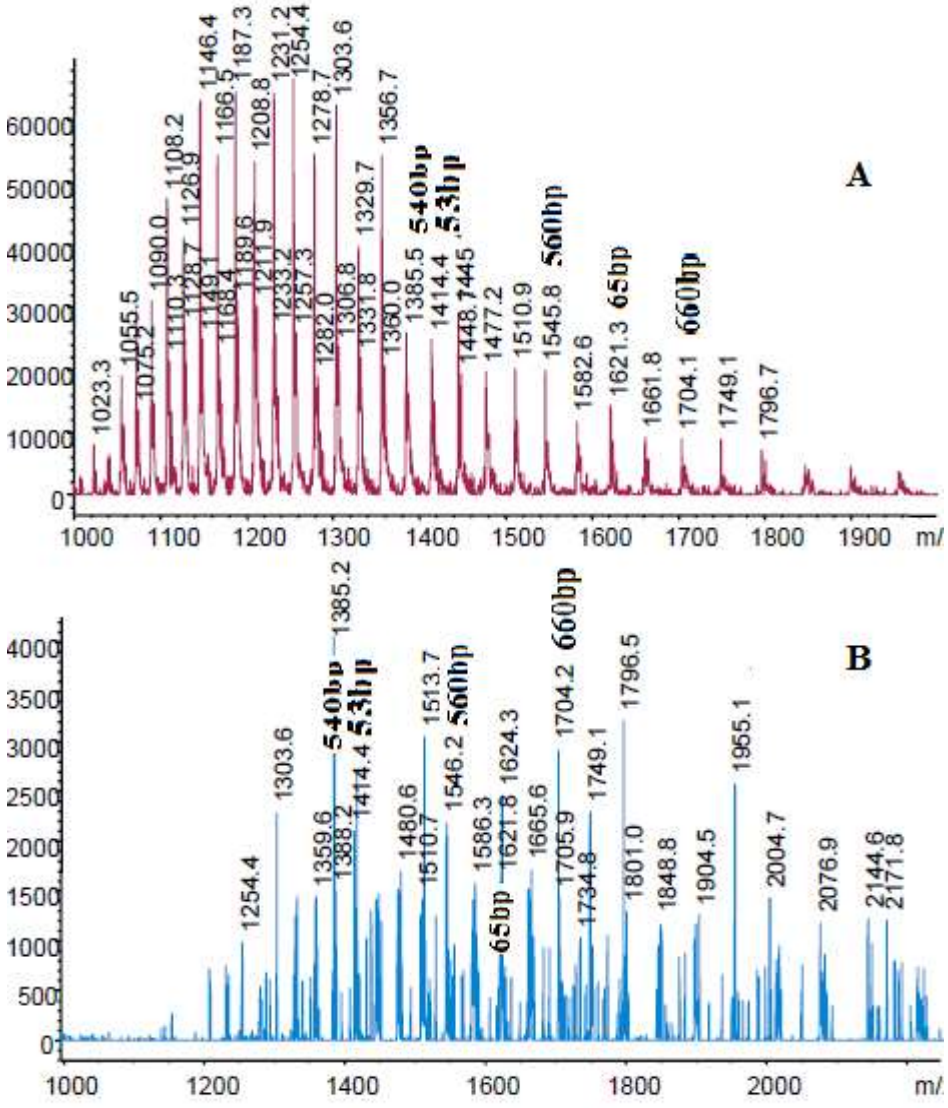
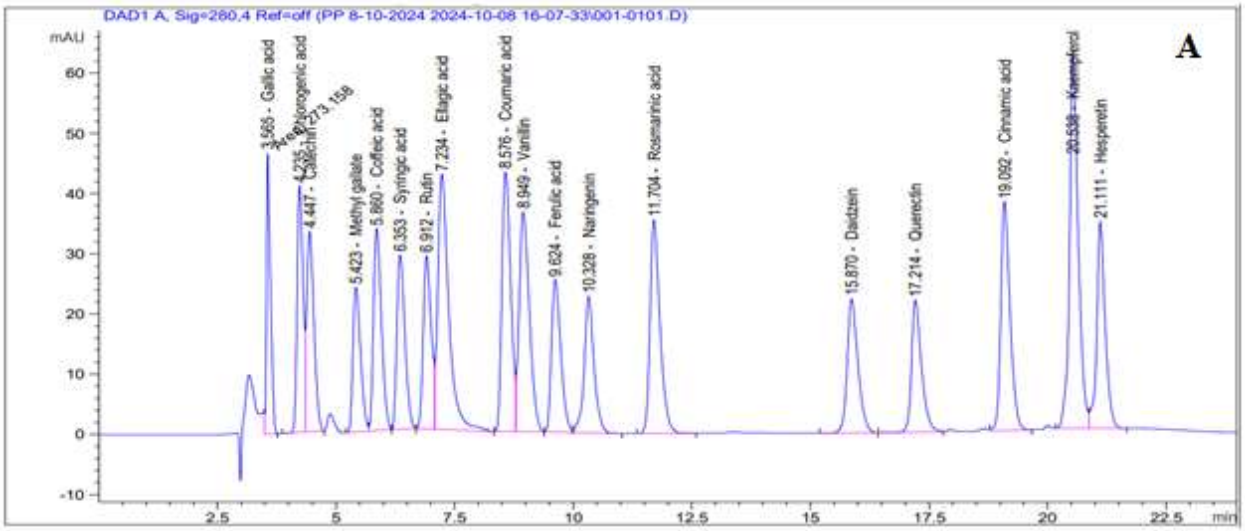


Fig. (3): HPLC spectra showing the ion peaks of the mushroom proteins: A) standard; B) Sample

3.2.2 Total phenolic compounds

According to HPLC analysis (Figure 4, Table 2), they contain the following compounds: gallic acid, chlorogenic acid, catechin, caffeineic acid, syringic acid, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, propyl gallate, 4.7-Dihydroxyisoflavone, querectin, and cinnamic acid. These compounds may have contributed to their potential as medications. Several studies have demonstrated that several types of mushrooms contain antioxidant qualities. Using 60% ethanol and water, extracts from *Agaricus bisporus* (white button mushroom) and *A. brasiliensis* (Brazilian button mushroom) were tested for antioxidant activity, total polyphenol content, and flavonoid content. (**Jan et al. 2013**). The amount of polyphenols in the A. bosporus aqueous extract was greater. The total flavonoid content of both A. brasiliensis and A. bisporus extracts was greater. **(Kanok-Orn *et al.*, 2009)**. 95% ethanol and aqueous extracts of two different kinds of mushrooms—Pleurotus ostreatus and P. sajor-caju—obtained from a neighbouring Thai farm were found to have antioxidant properties. The aqueous extracts of both mushrooms showed the highest quantity of total polyphenols and higher antioxidant activity when compared to the ethanol extracts. Gina tested methanolic extracts of P. sapidus, P. ostreatus, and P. sajor-caju fruiting bodies and mycelium for total polyphenol content and antioxidant activity. The highest levels of antioxidant activity and reducing power were frequently found in the fruiting bodies. **(Jeen *et al.*, 2014).**



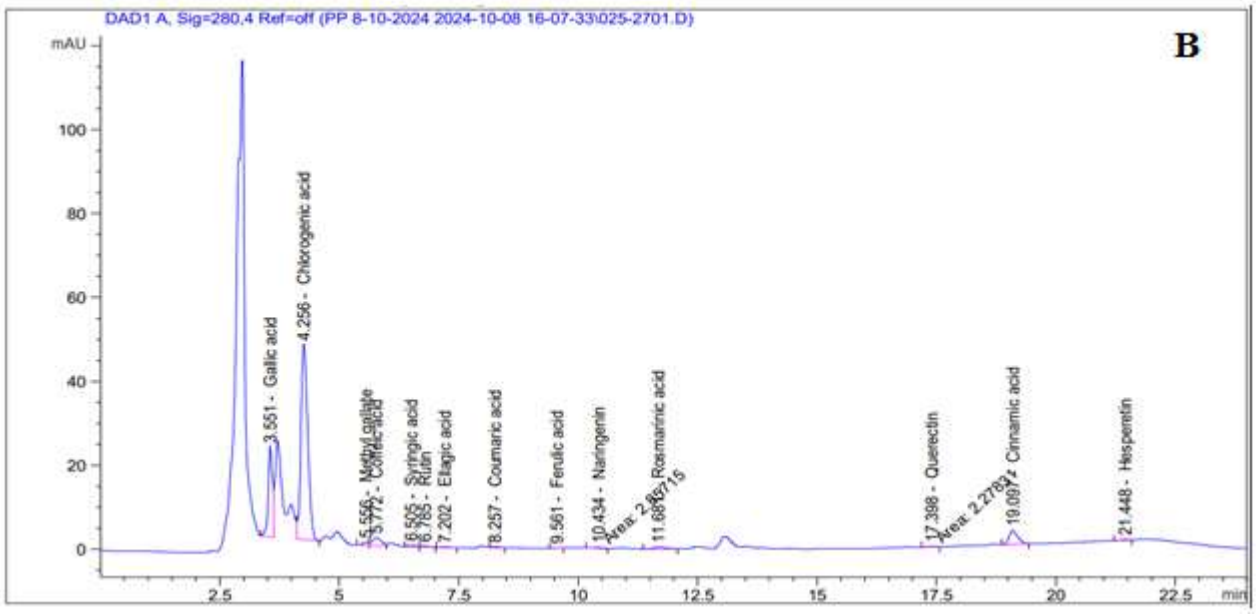


Fig. (4): HPLC chromatogram: A) Standard mixture of polyphenolic compounds; B) ethanlic extract of mushrooms.

Table 2: polyphenolic compounds of mushrooms

Sample	Area	Conc. (µg/mL)	Conc. (µg/g)
Gallic acid	135.28	9.90	495.23
Chlorogenic acid	465.38	64.85	3242.46
Catechin	0.00	0.00	0.00
Methyl gallate	6.22	0.35	17.40
Coffeic acid	24.96	1.28	64.03
Syringic acid	3.12	0.18	9.17
Rutin	2.78	0.42	20.82
Ellagic acid	1.80	0.18	9.12
Coumaric acid	3.49	0.13	6.28
Vanillin	0.00	0.00	0.00
Ferulic acid	0.89	0.05	2.59
Naringenin	2.86	0.26	13.18
Rosmarinic acid	9.46	0.92	45.93
Daidzein	0.00	0.00	0.00
Querectin	2.28	0.28	14.19
Cinnamic acid	41.24	0.80	39.96
Kaempferol	0.00	0.00	0.00
Hesperetin	1.58	0.07	3.70

3.3 Biological activities:

3.3.1 Antioxidant activity

Antioxidant activity is assessed using the DPPH radical scavenging technique. Fungi and phytochemicals have also been regarded as sources of physiologically active compounds that can be employed to prevent disease and lessen oxidative damage in humans. Numerous metabolites, such as beta-carotene, vitamin C, and vitamin E, as well as phenolic compounds—which are well-known for being potent antioxidants—are

produced by fungi. (**Gan et al., 2013)**. According to this viewpoint, dietary antioxidants are becoming more and more significant as possible defence mechanisms that lessen oxidative damage. The antioxidant qualities of mushrooms in particular have been investigated, and a number of antioxidant chemicals, including phenolic compounds, have been found from these sources. The outcome was 16.1µg/mL (IC50). Table 3 provides a summary of the polyphenol-polysaccharide-protein complex 450's oxidation activity. **(Gan *et al.*, 2013).**

Table 3. Evaluation of antioxidant activity in mushrooms

(Conc. µg/mL)	DPPH scavenging%	
	Extract	**Ascorbic Acid St.**
1000	93.9±0.004	97.9±0.003
500	88.8±0.003	96.3±0.002
250	80.9±0.004	93.9±0.001
125	73.8±0.006	90.8±0.002
62.5	65.8±0.006	83.3±0.002
31.25	57.2±0.005	74.0±0.002
15.625	48.6±0.003	65.7±0.002
7.8125	42.0±0.003	57.7±0.004
3.9	34.5±0.007	51.5±0.003
1.95	26.3±0.004	41.7±0.003
0	0.0±0.003	0.0±0.003
IC_{50}	**16.1**	**3.07**

3.3.2 Cytotoxic activity

The present study was conducted to evaluate the cytotoxic activity of effect against caco2, Hela and vero cells. The data presented in (Figure 5, Table 4) show a summary of the results of the mushroom extract used in this study. The effect on normal cells was low (IC50 ٧٤,٦٥ µg/mL), the effect on liver cancer cells was moderate (IC_{50} 56.2 µg/mL), and the effect on colon cancer cells was high (IC_{50} 23.1 µg/mL).

Anti-tumor activity assessed its impact on human cervical adenocarcinoma (HeLa) cells as well as other cancer cell lines. PSK or medium alone was used to cultivate tumour cell lines. In cancer cell lines, proliferation was shown to be inhibited. For HeLa cells, the inhibition rate (57%) was higher at lower PSK concentrations (50 µg/mL vs. 100 µg/mL) compared to the control. HeLa cells partially accumulated in the G0/G1 phase, whereas the number of cells in the S and G2/M phases decreased, according to a cell cycle phase distribution analysis. 36% of PSK-treated human gastric cancer cells had detectable active caspase-3 protease; HeLa cells did not exhibit this effect. **(Jimenez-Medina *et al.*, 2008)**. This study shown that mycelium extracts had a greater impact on HeLa, human colon cancer, and human lung adenocarcinoma cell lines than basidiocarp extract did. The extracts had the greatest effect on the HeLa cells. **(Knezevic *et al.*, 2018).**

Table 4. Effect of Polyphenolic- Polysaccharides –Protein Complex against caco2, Hela and vero cells

Sample	Conc. µg/mL	Viability %	Toxicity %	IC_{50}	Viability %	Toxicity %	IC_{50}	Viability %	Toxicity %	IC_{50}
		Caco2			Hela			Vero		
Extract		100	0	23.1 ± 0.27	100	0	56.2 ± 0.92	100	0	74.6 ± 0.71
	250	3.84	96.15		5.57	94.42		7.35	92.64	
	125	6.48	93.51		18.70	81.29		24.45	75.54	
	62.25	7.16	92.83		45.87	54.12		50.47	49.52	
	31.25	22.02	77.97		72.50	27.49		78.06	21.93	
	15.62	76.99	23.00		99.72	0.27		99.14	0.85	
	7.81	100	0		99.90	0.09		99.77	0.22	
Doxo.	250	3.26	96.73	16.12 ± 0.16	5.79	94.20	24.74 ± 0.29	5.60	94.39	27.94 ± 0.17
	125	3.16	96.83		10.96	89.03		8.34	91.65	
	62.25	5.50	94.49		19.70	80.29		11.84	88.15	
	31.25	12.76	87.23		32.11	67.889		41.27	58.72	
	15.62	38.49	61.50		75.27	24.72		82.36	17.63	
	7.81	79.82	20.17		96.01	3.98		99.68	0.31	

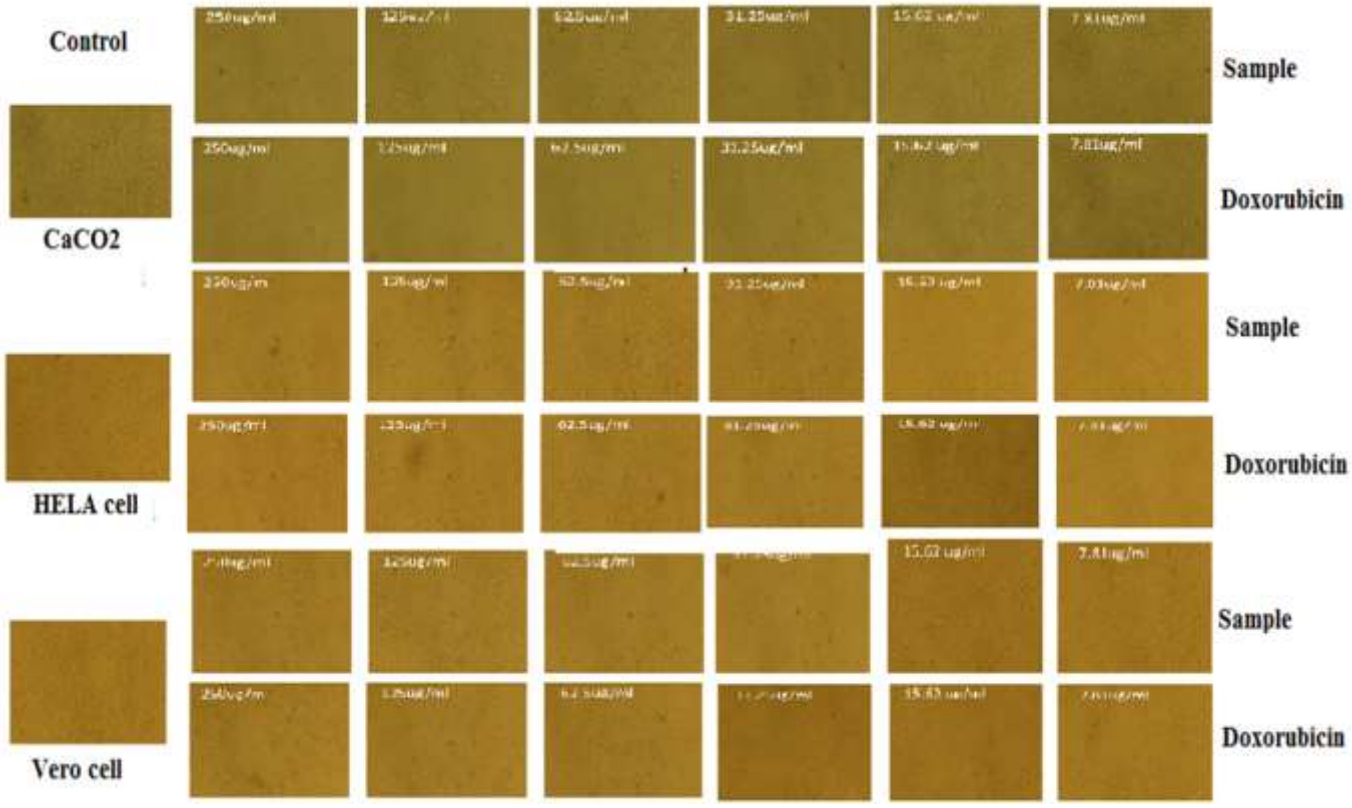


Fig. (5): Effect of a sample of doxorubicin on) Hela, Caco2 & Vero) cells at different concentrations.

4. Conclusion

According to in vitro studies, mushrooms, a common fungus, offer remarkable therapeutic potential and high nutritional value. Similar in nutritional value to vegetables, mushrooms of all kinds are a good source of fibre, protein, unsaturated fatty acids, carbs, and several essential vitamins. The culinary and nutritional benefits of edible mushrooms are widely recognized, but less is known about their possible medical use. It has been demonstrated that edible mushrooms have anti-oxidant and anti-cancer bioactivities. Determining the exact method of action of various biochemical formulations should be the main goal of future study. In summary, the study's findings have improved our understanding of the physiologically active ingredients in mushroom extract and powder. As a result, cancer patients may benefit from include mushroom powder and extract in their diet and medicine schedule.

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