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

Yasser A. Selim [1],*, Wesam A. Kollab[2], Akmal S. Gaballa[1], Rehab E. Tag[1] and Amira M. Mohamed[1]

[1]Faculty of Specific Education, Zagazig University, Zagazig, 44519, Egypt

[2]Department of Chemistry, Faculty of Science, Alasmarya Islamic University, Zliten, Libya

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Yasser A. Selim
Faculty of Specific Education, Zagazig University, Zagazig, 44519, Egypt

Wesam A. Kollab
Department of Chemistry, Faculty of Science, Alasmarya Islamic University, Zliten, Libya

Akmal S. Gaballa
Faculty of Specific Education, Zagazig University, Zagazig, 44519, Egypt

Rehab E. Tag
Faculty of Specific Education, Zagazig University, Zagazig, 44519, Egypt

Amira M. Mohamed
Faculty of Specific Education, Zagazig University, Zagazig, 44519, Egypt

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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 74.65 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 ($IC_{50} \pm 23.1$µg/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 الطبعية بنسبة بسيطة.

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

1. Introduction:

The majority of the cancer treatments are accompanied by a degree of herbal supplements. There is advantageous effect of medicinal plants on cancer. Several therapies include herbal remedies to improve the quality of life. Plant derived natural products such as flavonoids, terpenes, alkaloids etc. have received wide attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemo preventive effects (Babu et al., 2002). The look for anti-cancer

agents from plant sources started in the 1950s with the discovery and development of the vinca alkaloids like vincristine and vinblastine, and the isolation of the cytotoxic podophyllotoxines. Natural products discovered from medicinal plants have played a vital role in the management of cancer. Natural products or natural product derivatives consist of 14 of top 35 drugs in 2000 based on worldwide sales (Butlet, 2004). Plant based medication has definitely found a role in cancer healing (chemotherapy), and the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively. In particular, there is growing interest in the pharmacological estimation of various plants used in Indian tradition system of medicine. There are more than 2, 70,000 higher plants existing on this planet. But only a small portion has been surveyed phytochemically. So, it is anticipated that plants can provide potential bioactive compounds for the development of new leads to combat cancer diseases (**Shoeb, 2006**).

Humanity continues to suffer the scourge of cancer, a disease that is associated with uncontrolled cell growth. In 2013, it was reported to be among the leading causes of death, second to cardiovascular diseases. It is estimated that death due to cancer will rise to thirteen million in 2030 **(Ferlay *et al.*, 2008).** The fight against cancer has intensified in the past decades with multidirectional approach including behavioral and dietary change, chemotherapy, radiotherapy, surgery, and recently immunotherapy. Unfortunately, these approaches are not void of serious side effects spanning from recurrence and weakened immune system to reduced quality of life (QoL) of patients. This has raffled scientists, leading to concerted efforts of finding better therapies that, apart from managing the cancerous cells, boost the immune system to fight cancer and other diseases **(Chen et *al.*, 2004).** Among these therapies, complementary and alternative medicine (CAM) has been fronted as an alternative due to its potential of holistic treatment including augmenting the immune system. Many CAMs are plant-derived, including algae and mushrooms that have been used widely in many parts of the world, where they are regarded as biological response modifiers (BRMs) and immunoceuticals **(Kidd. 2000).** Mushroom is one of the natural resources that nature has given us to maintain a good healthy lifestyle for a better life. Therefore, mushrooms have been used as food and medicine for thousands of years The data presented in (Figure 1) also show some medicinal fungi with anti-cancer potential **(Kumar *et al.*, 2021).**

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

The polysaccharides present in mushrooms have attracted much attention in recent times due to their anti-cancer and immunomodulatory properties. In contemporary medical and pharmaceutical research, immunomodulators are the factors that are mainly focused on **(Damini *et al.*, 2018).** Mushrooms are named after the spore-producing reproductive structure they contain. Older classifications placed fungi in the kingdom Plantae, but current classification recognizes fungi as a separate group of organisms under the kingdom Fungi. This is due to the presence of chitin in their cell walls. A mushroom is a fleshy, spore-bearing fruiting body. Mushrooms are typically produced above ground on soil or its substrate, primarily by the basidiomycetes and ascomycetes. Although wild mushrooms are seasonal and can be collected and used, they can be hybridized by culturing spores or tissues in laboratories **(Borchers *et al.*, 2008).** There are many edible and poisonous mushroom species that are cultivated annually. Of the 1700 species of mushrooms worldwide, only 100 are edible. Three species are commonly cultivated. Only three are commonly grown: white button mushrooms (Agaricus bisporus L.), oyster mushrooms (Pleurotus ostreatus L.), and paddy straw mushrooms **(Kumar *et al.*, 2021).** Chitin and beta-glucan are two important

substances found in the cell walls of mushrooms. They are important for health and the treatment of many diseases because they contain beta-glucans β (1→3), β (1→4), and β (1→6). In addition to these substances, mushrooms also contain other important elements. Among them are polysaccharides, protein terpenoids, agartin, ergosterol, selenium, and polyphenols. Protein-polysaccharide complexes. In addition to their medicinal properties, these substances are usually thought to be medicinal properties, biological response modifiers (BRMs) **(Shiu-Nanc *et al.*, 2014).**Polysaccharides are carbohydrate molecules composed of long chains of monosaccharides linked together by glycosidic bonds. When hydrolyzed, they yield monosaccharides and oligosaccharides. Polysaccharides can be found in a variety of materials, including plants, microbes, algae, and animals. Organic materials contain a large number of reactive functional groups, a diverse chemical composition, and a wide range of molecular weights, all of which contribute to their structural and physical diversity. Modification of the many reactive groups on the polysaccharide chains can lead to the formation of various polysaccharide derivatives. These derivatives have different physical and chemical properties than the monosaccharide units, depending on their structure. They are insoluble in water and have an amorphous structure **(Devi N *et al.*, 2017).**

Proteins are polymers of amino acids covalently linked together by peptide bonds in a chain. Proteins are the most useful macromolecules in living organisms because they play many vital functions in all biological processes. They act as catalysts, generate movement, transport and store oxygen, transmit nerve impulses, provide mechanical support and immune protection, and control growth. Most proteins are linear polymers made from a chain of up to 20 different types of L-α-amino acids. All protein amino acids have common structural units. Protein structure is usually defined at four levels of complexity: primary, secondary, tertiary, and quaternary structure.The activity of polysaccharides is determined by their conformation, composition, and size **(Zhang *et al.*, 2007).** Although chitin and cellulose are among the other polysaccharides found in fungal cell walls, glucose, or glucose polymers, is the most important of these carbohydrates. These carbohydrates form a variety of glycosidic bonds, including A or B configurations in various positions (e.g., B-(1!3), B-(1!6), B-(1!4), or 2), and A-(1!3) glucan or heteroglycan also show the polysaccharides in the primary cell wall of the fungus and some biological activities of beta-glucan.They are commonly associated with proteins to form protein-glucan complexes, such as glucomannan and galactomannan **(Ooi *et al.*, 2000).**

Considered as the polysaccharides with the highest biological activity, beta-glucans which are homopolymers of D-glucose are the most abundant carbohydrates in the cell walls of many microorganisms, such as fungi, yeast, algae, bacteria, lichen and plants, and exhibit immunomodulatory, antitumor and anti-infective activities. Therefore, they are clinically used for immuno-oncology in many countries in the world **(Laura B *et al.*, 2011).** Studies have investigated the immunomodulatory effects of polysaccharides in mushrooms. One of the main goals of current and advanced medical research is to discover new and safe drugs with minimal or no side effects. In the past 20 years, research on polysaccharides has increased dramatically; the most studied biological activities of these polysaccharides are immunomodulation, anticancer and tumor inhibition. Clinical studies have suggested that nutritional preparations obtained from medicinal mushrooms are natural medicines with very few side effects. Polysaccharides in mushrooms are emerging new agents that can enhance immune responses. The efficacy of a drug obtained from any natural resource depends only on the dosage, concentration, purification methods and duration of treatment. The medicinal properties of different types of mushrooms have been identified. Therefore, the present study aimed to detect the polyphenolic polysaccharide-protein complex present in mushroom powder and extract as antioxidants that showed their effect on cancer cells.

2. Materials and Methods

2.1 Materials

2.1.1 Plant material

Edible Mushroom (Toadstool & Mushroom) was obtained from the local market in Zagazig City, Governorate, Sharkia, Egypt, and were dried and ground to obtain a fine powder. The plant material was documented at the Central Laboratory for Breeding, Food and Feed, Faculty of Technology, Zagazig University, Egypt. A voucher sample was deposited (voucher number 2024/5/151). Sigma Aldrich Chemical Co., St. Louis, Mo, U.S.A., was the source of the following chemicals. All chemicals and reagents used in this study are of highest analytical grade.

2.2 Methods

2.2.1 Preparation of mushroom powder and extracts:

Themushrooms were dried in a clean room at room temperature for 4 days. Then the mushroom material was ground in a blender to obtain a powder and stored in tightly closed glass bottles in a dry place until use (**Russo, 2001),** who reported that herb is best kept in a dry and dark location to reduce oxidation of their contents. 50 g of mushroom powder was soaked in 500 ml of 96 %ethanol to obtain the extract.

2.2.2 Extraction of Polyphenolic-Protein-Polysaccharide Complexes:

Obtaining and purifying 200 g of dried plant material were chopped, suspended in 2 l of Ethanol. The ethanol fraction was then eliminated by filtering. The new portion of ethanol was used to repeat the process. A clear supernatant was then obtained by centrifuging the plant residue at 8000 rpm for 15 minutes at room temperature, after it had been suspended in 2 l of 0.1 M NaOH and refluxed for 3 hours at 80 °C. After neutralizing the alkaline fraction with 1 M HCl, the dry crude plant extract 30% of the dried plant material was concentrated on a rotary evaporator under decreased pressure. One litre of water was used to dissolve the extract, followed by one liter of diethyl ether, at 34 °C. Following that, the water-soluble fraction was separated from the organic one and collected. The new diethyl ether part was used to repeat the process. The combined water extract was then mixed with 1 liter of chloroform. For six hours, the mixture was refluxed at 61 °C. After repeating the chloroform extraction procedure, the water-soluble fraction was refluxed twice using a 3:1 ethanol and chloroform mixture for six hours at 70 °C. Following a multi-step extraction procedure using organic solvents, the water-soluble fraction was dried off using a rotary evaporator operating at lower pressure. Following a 24-hour stirring period at room temperature, it was suspended in 500 milliliters of methanol and subsequently filtered out. This process was carried out five times. After drying at room temperature and dissolving it in distilled water, the precipitate was dialyzed against water for six days **(Wu DT *et al.*, 2020).**

2.2.3 Chemical components of mushroom powder:

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 HPLC analysis of phenolic compounds

For polyphenolic, HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse plus C8 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40 °C.

For protein, column: Poroshell 300SB-C18, 2.1 x 75 mm, 5 µm Mobile Phase Gradient: 20-100% B in 5.5 min. A: water + 0.1% TFA or

FA B: ACN + 0.1% FA or TFA Flow Rate: 500 µL/min Temperature: 60°C Injector: 1 µL Sample: 10 pmol of BSA Electrospray ionization: positive ion Vcap: 6000V Drying Gas: 12L/min 350ºC Nebulizer: 45 psi Scan: 600-2500 amu Step size: 0.15 amu Peak width: 0.06 min

2.2.5 Morphological assay

Large-scale, morphological changes that occur at the cell surface, or in the cytoskeleton, can be followed and related to cell viability. Damage can be identified by large decreases in volume secondary to losses in protein and intracellular ions of due to altered permeability to sodium or potassium.Necrotic cells: nuclear swelling, chromatin flocculation, loss of nuclear basophilia. Apoptotic cells: cell shrinkage, nuclear condensation, nuclear fragmentation. **(Alley *et al.*, 1988), (Slater et al., 1963)**

2.2.6 Evaluation of antioxidant activity by DPPH radical scavenging method

Free radical scavenging activity of different extracts of leaves plant was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/ml). Here, only those extracts are used which are Solubilize in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. By using spectrophotometer (UV-VIS milton roy). Reference standard compound being used was ascorbic acid and experiment was done in triplicate.16 The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity **(Gonzalez et al., 2016).**

2.2.7 Determination of sample cytotoxicity on cells (MTT protocol)

96 well tissue culture plates was inoculated with 1 X 10^5 cells / ml (100 ul / well) and incubated at 37°C for 24 hours to develop a complete monolayer sheet. Growth medium was decanted from 96 well micro titer plates after confluent sheet of cells were formed, cell monolayer was washed twice with wash media. Two-fold dilutions of tested sample were made in RPMI medium with 2% serum (maintenance medium). 0.1 ml of each dilution was tested in different wells leaving 3 wells as control, receiving only maintenance medium. Plate was incubated at 37°C and examined. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation. MTT solution was prepared (5mg/ml in PBS) (BIO BASIC

CANADA INC). 20ul MTT solution was added to each well. Place on a shaking table, 150rpm for 5 minutes, to thoroughly mix the MTT into the media. Incubate (37C, 5% CO2) for 4 hours to allow the MTT to be metabolized. Dump off the media. (Dry plate on paper towels to remove residue if necessary.Resuspend formazan (MTT metabolic product) in 200ul DMSO. Place on a shaking table, 150rpm for 5 minutes, to thoroughly mix the formazan into the solvent. Read optical density at 560nm and subtract background at 620nm. Optical density should be directly correlated with cell quantity **(Alley *et al.*, 1988), (Slater et al., 1963) and (Van de loosdrecht *et al.*, 1994).**

2.2.8 Extraction of Polyphenolic-Protein-Polysaccharide Complexes

Protein-polysaccharide natural fractions varying in protein and polysaccharide content were obtained by sequence aqueous extraction processes which make foaming with water.

In general, other stabilizing agent's polysaccharides being the most popular must be added in order for proteins to produce stable foams. The food sector usually uses polysaccharides because of their texturing qualities as thickening and gelling agents. Their characteristics as proteins have mostly been researched without considering how they interact with other substances. Nonetheless, polysaccharides and proteins frequently interact, and polysaccharide combinations typically exhibit synergistic qualities. For example, guar and xanthan combinations have strong gelling qualities, however when employed separately as thickening agents in food, guar and xanthan often don't have gelling qualities. In the past ten years, the food industry has become more interested in the theoretical knowledge of the stability of protein–polysaccharide mixes in water (**Narchi *et al.*, 2009).**

HPLC mechanism, (Fig.2) showed that Molecules are separated by size based on their ability to penetrate the pores of the column support. Multiple columns can be put together with different pore sizes to extend the separation range. Which molecules separate in the linear range depends on the pore size of the packing. This elucidated that, HPLC is the most suitable for investigate the protein mixture which was detected the presence of protein 540 with high concentration as shown in (Fig 3). Protein 540 represented as the most potent cytotoxic agent for cancer cells **(Elhusseiny *et al.*, 2021).**

The p53 protein is a transcription factor known as the "guardian of the genome" because of its critical function in preserving genomic integrity. The *TP53* gene is mutated in approximately half of all human malignancies, including those of the breast, colon, lung, liver, prostate, bladder, and skin. When DNA damage occurs, the *TP53* gene on human chromosome 17 stops the cell cycle. If p53 protein is mutated, the cell cycle is unrestricted and the damaged DNA is replicated, resulting in uncontrolled cell proliferation and

cancer tumours. Tumor-associated p53 mutations are usually associated with phenotypes distinct from those caused by the loss of the tumor-suppressing function exerted by wild-type p53protein. Many of these mutant p53 proteins have oncogenic characteristics, and therefore modulate the ability of cancer cells to proliferate, escape apoptosis, invade and metastasize. Because p53 deficiency is so common in human cancer, this protein is an excellent option for cancer treatment **(Marei *et al.*, 2021)**.

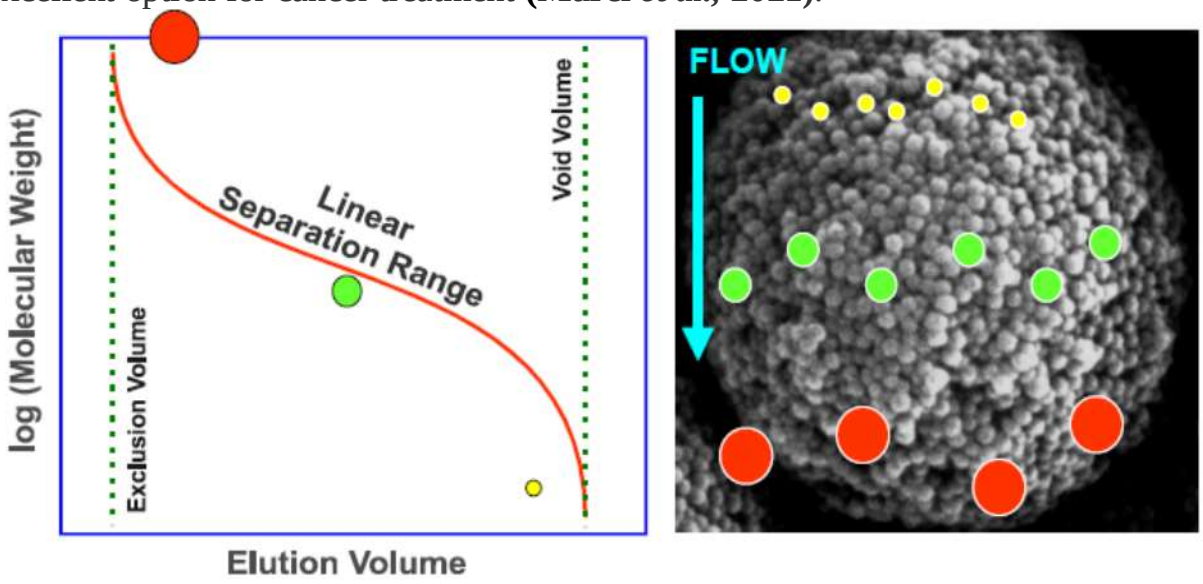

Fig. (2): HPLC Mechanism of the mushroom proteins

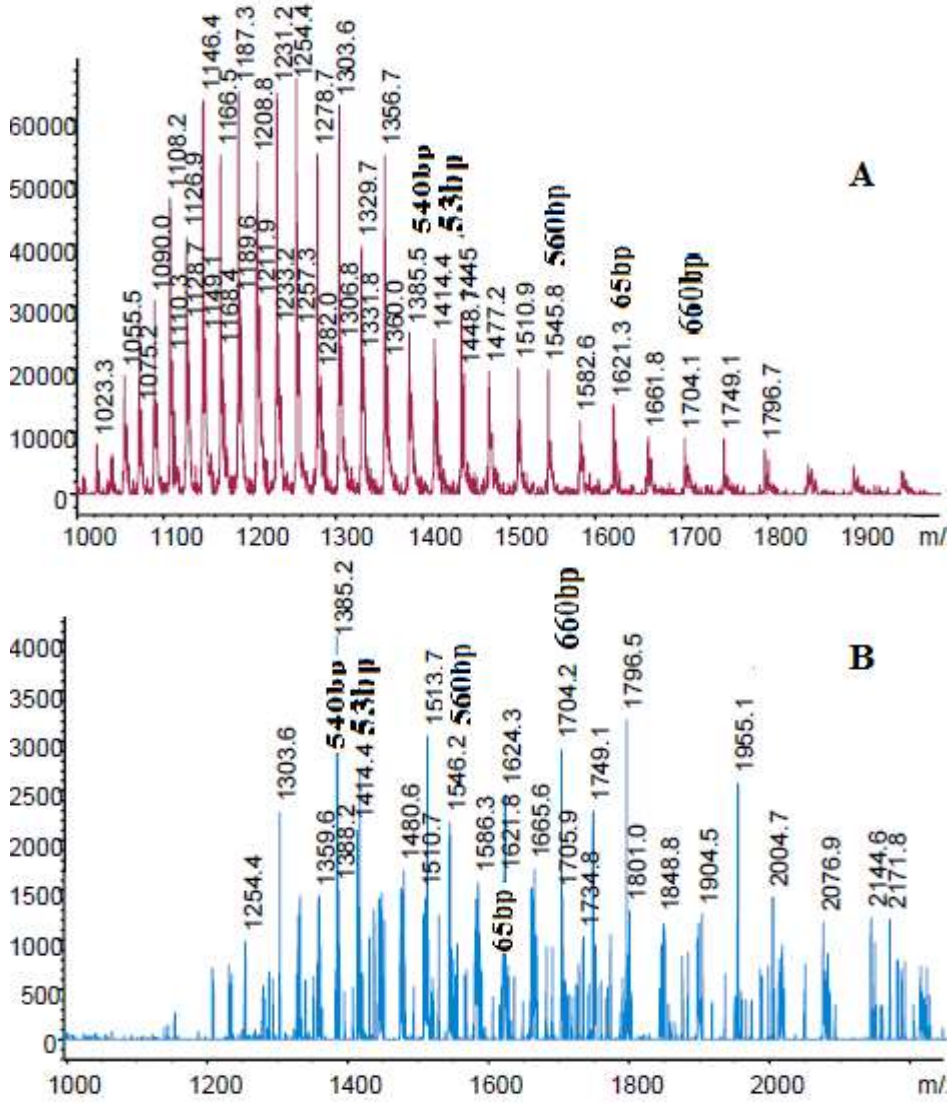

Fig. (3): HPLC spectra showing the ion peaks of the mushroom proteins: A) standard; B) Sample

3. Results and Discussion

3.1 Chemical composition

All the results of Chemical composition analysis are shown in (Table 1). Summary of the results of the dried mushroom powder used in this study, which contains a large amount of protein (3809), carbohydrates (23.82).

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 Total phenolic compounds

HPLC analysis indicate the presence of Gallic acid, Chlorogenic acid, Catechin, Coffeic acid, Syringic acid, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Propyl Gallate, 4`.7-Dihydroxyisoflavone, Querectin and Cinnamic acid (Figure 4, Table 2**)** that might have been responsible for their therapeutic potential. Several studies have demonstrated the antioxidant activity of different mushroom species. Jan et al. (2013) measured the total polyphenol and flavonoid content, as well as the antioxidant activity of extracts obtained using 60% ethanol and water from Agaricus bisporus (white button mushroom) and A. brasiliensis (Brazilian button mushroom). The aqueous extract of A. Bosporus had higher polyphenol content. Both A. brasiliensis extracts showed a higher total flavonoid content than A. bisporus extracts. Pornariya and **(Kanok-Orn *et al.*, 2009)**. Investigated the antioxidant properties of aqueous and 95% ethanol extracts of two mushroom species, Pleurotus ostreatus and P. sajor-caju, obtained from a local farm in Thailand. The aqueous extracts showed the highest amount of total polyphenols and better antioxidant activity than the ethanol extracts of both mushrooms. Gina investigated the antioxidant activity and total polyphenol content of methanolic extracts of the mycelium and fruiting body of P. sajor-caju, P. ostreatus and P. sapidus. In general, the fruiting bodies showed the highest antioxidant activity and reducing power **(Jeen *et al.*, 2014).** These results are consistent with the current study.

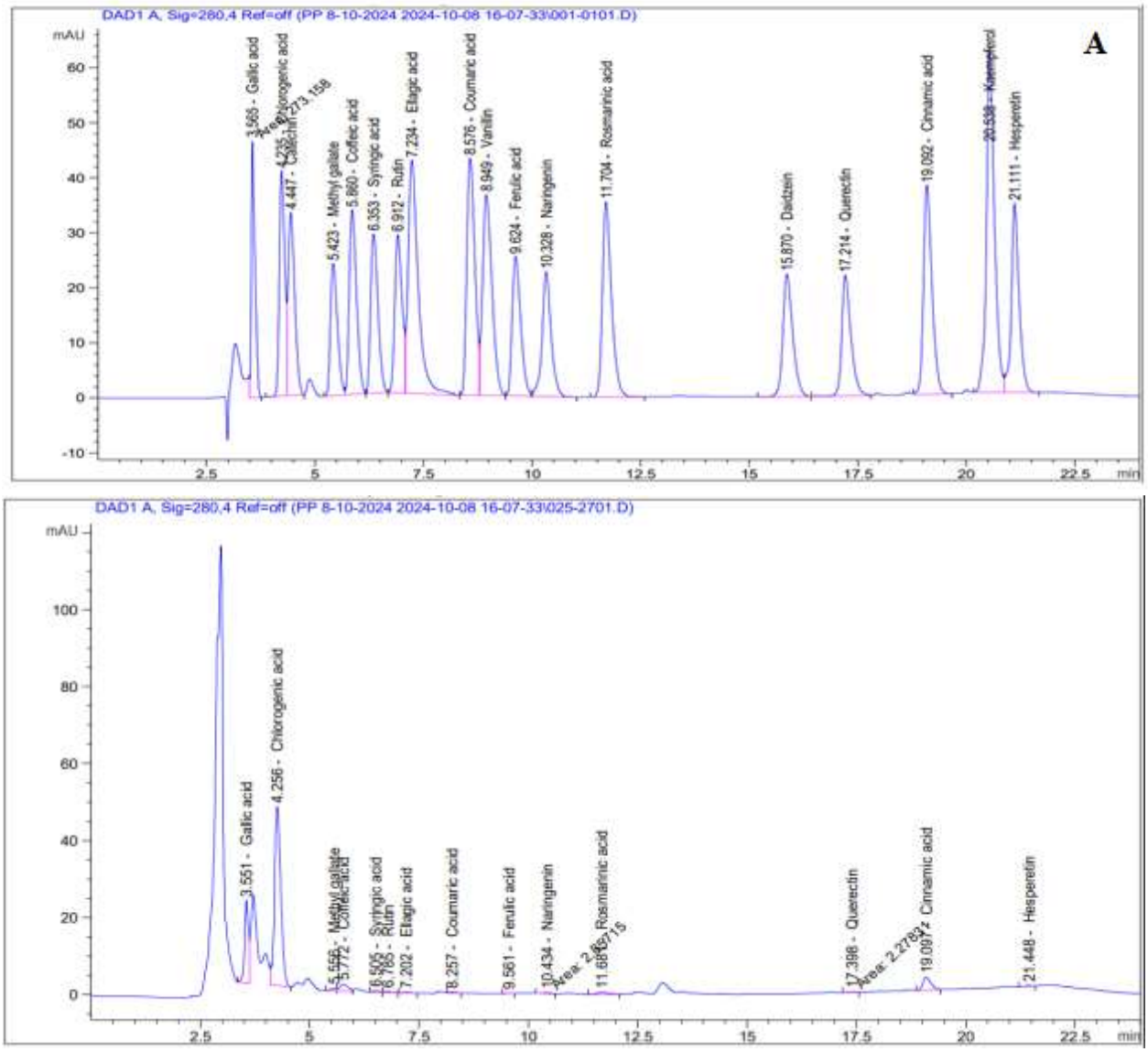

Fig. (4): HPLC chromatogram: A) Standard mixture of polyphenolic compounds; B) ethanlic extract of mushrooms.

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

Evaluation of antioxidant activity by DPPH radical scavenging method. Phytochemicals, fungi have also been considered as a source of biologically active substances that can be used to reduce oxidative damage in humans and are useful in disease prevention. Fungi produce a large amount of metabolites, including vitamin C, vitamin E, and beta-carotene, as well as phenolic compounds, which are known to be excellent antioxidants (**Gan et al., 2013)**. From this perspective, antioxidants in the diet are gaining importance as potential protective agents that reduce oxidative damage. In particular, the antioxidant properties of mushrooms have been studied, and several antioxidant compounds extracted from these sources, such as phenolic compounds, have been identified. The result (IC_{50} 16.1µg/mL). Was as shown in the (table 3) give an overview of the oxidation activity of the polyphenol-polysaccharide-protein complex 450 **(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 74.65 µg/mL), 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} 23.1 µg/mL).

Anti-Tumour activity evaluated its effect on various tumour cell lines, including human cervix adenocarcinoma (HeLa) cells. Tumour cell lines were cultured with PSK or in medium alone. Inhibition of proliferation was demonstrated in tumour cell lines. In the case of HeLa cells, the inhibition rate (57%), in correlation with the control, was higher at a lower concentration of PSK (50 µg/mL vs. 100 µg/mL). Cell cycle phase distribution analysis showed partial accumulation of HeLa cells in the G0/G1 phase and a decreased number of cells in the S phase and G2/M phase. In human gastric cancer cells, detectable active caspase-3 protease was present in 36% of PSK-treated cells; this effect was not found in HeLa cells **(Jimenez-Medina *et al.*, 2008)**. Knežević et al. demonstrated the antitumour effect of C. versicolor on HeLa cells **(Knezevic *et al.*, 2018).** This work showed a stronger effect from mycelium extracts than basidiocarp extract on HeLa, human colon carcinoma, and human lung adenocarcinoma cell lines. The HeLa cells were the most sensitive to the extracts **(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	

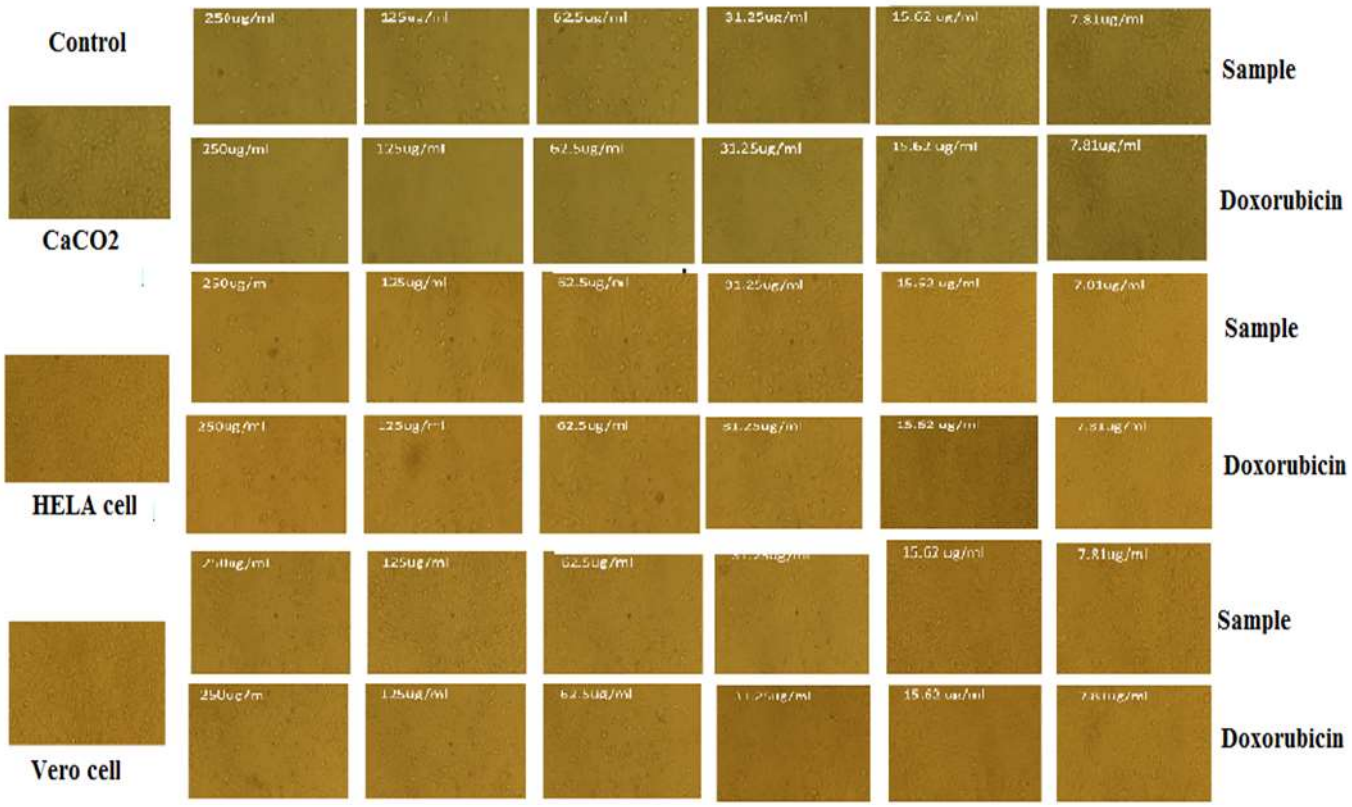

Fig. (5): Effect of a sample of doxorubicin on) Hela, Caco2 & Vero) cells at different concentrations

4. Conclusion

In vitro research has shown that mushrooms, a fungus that is widely available, have amazing medicinal potential and excellent nutritional value. All types of mushrooms are a good source of carbohydrates, protein, unsaturated fatty acids, some important vitamins, and fibre from the diet, all of which are nutritionally similar to vegetables. Although edible mushrooms are well known for their culinary and nutritional benefits, little is known about their medicinal potential. The bioactivities of edible mushrooms have been shown to include anti-oxidant and anticancer. Future research should focus on figuring out the precise mode of action of several biochemical formulations. In conclusion, the results of the study have increased the current knowledge of the biologically active components in mushroom powder and extract. Therefore, the consumption of mushroom powder and extract in the diet and medication regimen may be beneficial for patients with cancer.

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