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Potential Cytotoxic activity of *Ganoderma lucidum* Extracts on Human Skin Squamous Carcinoma Cell Line

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Abstract

The central focus of this investigation is examining the cytotoxic properties of various crude extracts derived from *Ganoderma lucidum* (*G. lucidum*) on human skin squamous carcinoma cells in vitro. The present study centres on three distinct extracts, namely, hot aqueous, cold aqueous, and ethanolic, all of which were prepared from *G. lucidum* sourced from DXN Company. The primary objective is to elucidate the potential inhibitory effects these extracts have on the proliferation of human skin squamous carcinoma cells. To achieve this goal, the extracts were subjected to analysis using GC-MS technology. The colorimetric assay method also played a critical role in assessing cytotoxicity (inhibition rate) and cellular proliferation (proliferation rate). The study involved preparing eleven dilute solutions in a two-fold series, resulting in concentrations ranging from 7.8 to 4000 µg/ml, which were then applied to the skin squamous carcinoma cell line (passage 27) over 24, 48, and 72 hours. The analysis revealed that *G. lucidum* predominantly contains organic compounds like alcohols, aldehydes, esters, and ketones. The cytotoxic impacts observed in the skin squamous carcinoma cell line were significantly influenced by the type of extract, its concentration, and the exposure time. Specifically, the higher concentrations (4000 µg/ml) of all three extracts demonstrated a significant inhibition ($P \leq 0.05$) of cancer cell growth in comparison to the control group (100% viability). Conversely, lower concentrations (7.8-26.5 µg/ml) were found to significantly stimulate ($P \leq 0.05$) the proliferation of skin cancer cells when compared with the control (viability 100%). The identification and assessment of bioactive compounds in the three *G. lucidum* extracts suggest these extracts have the potential to inhibit the growth of skin cancer cells.

Keywords: *Ganoderma lucidum*, Cytotoxicity, Inhibitory Rate, Proliferation Rate, HSSCC.

التاثير السام المحتمل لمستخلصات عش الغراب في الخط الخلوي لسرطان النسيج الحرشفي الجلدي

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الخلاصة

يركز هذا البحث بشكل أساسي على دراسة الخصائص السامة للخلايا للعديد من المستخلصات الخام المشتقة من فطر جانوديرما لوسيدوم (*G. lucidum*) على خلايا سرطان الجلد الحرشفي لدى البشر في

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المختبر. تركز الدراسة الحالية على ثلاثة مستخلصات مميزة، وهي المستخلص المائي الساخن والمستخلص المائي البارد والمستخلص الإيثانولي، وقد تم تحضيرها جميعًا من فطر جانوديرما لوسيدوم من شركة DXN. الهدف الأساسي هو توضيح التأثيرات المثبطة المحتملة لهذه المستخلصات على تكاثر خلايا سرطان الجلد الحرشفي لدى البشر. لتحقيق هذا الهدف، تم إخضاع المستخلصات للتحليل باستخدام تقنية GC–MS. لعبت طريقة التحليل اللوني أيضًا دورًا حاسمًا في تقييم السمية الخلوية (معدل التثبيط) وانتشار الخلايا (معدل الانتشار). تضمنت الدراسة تحضير أحد عشر محلولًا مخففًا في سلسلة مزدوجة، مما أدى إلى تركيزات تتراوح من 7.8 إلى 4000 ميكروجرام / مل، والتي تم تطبيقها بعد ذلك على خط خلايا سرطان الجلد الحرشفي (الممر 27) على مدار 24 و 48 و 72 ساعة. كشف التحليل أن *G. lucidum* يحتوي بشكل أساسي على مركبات عضوية مثل الكحولات والألدهيدات والإسترات والكيتونات. تأثرت التأثيرات السامة للخلايا التي لوحظت في خط خلايا سرطان الجلد الحرشفي بشكل كبير بنوع المستخلص وتركيزه ووقت التعرض. على وجه التحديد، أظهرت التركيزات الأعلى (4000 ميكروجرام / مل) لجميع المستخلصات الثلاثة تثبيطًا كبيرًا ($P \leq 0.05$) لنمو الخلايا السرطانية مقارنة بمجموعة التحكم (الحيوية بنسبة 100٪). وعلى العكس من ذلك، وجد أن التركيزات المنخفضة (7.8–26.5 ميكروجرام/مل) تحفز بشكل كبير ($P \leq 0.05$) تكاثر خلايا سرطان الجلد عند مقارنتها بالمجموعة الضابطة (قابلية البقاء 100%). يشير تحديد وتقييم المركبات النشطة بيولوجيًا في مستخلصات *G. lucidum* الثلاثة إلى أن هذه المستخلصات لديها القدرة على تثبيط نمو خلايا سرطان الجلد.

1. Introduction

Cancer has become a major public health issue worldwide. Since the mid-20th century, climate change has had a profound impact on human health. At the same time, the incidence of skin cancer is rising at an alarming rate, due to ozone depletion, heightened UV radiation, and global warming. Skin cancer is a severe and potentially life-threatening condition that can result from an imbalance between cell death and cell growth in the skin. Although UV radiation is the primary cause of the disease, other factors can contribute to the development, such as bacteria, metabolites in food and medicine, and genetic susceptibility [1, 2].

It remains the leading cause of death in developed countries, notably the United States and Australia and the second leading cause of death in developing nations, following closely behind heart disease. Cancer is responsible for a staggering one in six deaths worldwide. There were 39,068 diagnosed cancer cases reported among Iraqi nationals in 2022; Skin cancer is the eighth most common cancer in Iraq. 1415 cases, accounting for 3.6% of all cancer cases diagnosed in Iraq [3, 4, 5].

Cancer treatment drugs like cisplatin and cyclophosphamide can cause side effects, including nephrotoxicity, which may negatively impact a patient's quality of life. In addition to the harmful effects, the ability of some cancer cells to resist treatment has required the exploration of other methods. Therefore, chemotherapy fails to meet the treatment requirement. Throughout decades, *Ganoderma lucidum* (*G. lucidum*) has been used to enhance longevity and as a beneficial adjunctive therapy for cancer, owing to its little toxicity, no genotoxicity and efficacy in combination treatment. This substance operates by activating particular cellular processes such as the effect on immune cells or effect on tumor cells by cancer cell cycle arrest and apoptosis, distinguishing it from conventional chemotherapy drugs [6].

Ganoderma lucidum, commonly known as "Lingzhi or Reishi," is a mushroom believed to have longevity and health-promoting properties. It is a large, dark mushroom with a glossy exterior and a woody texture (Figure 1). It has been utilized for centuries, with a history spanning over 2000 years, in Traditional Chinese medicine, owing to its diverse array of

therapeutic properties, including its ability to combat tumors, allergies, viruses, liver damage, oxidative stress, high blood pressure, and inflammation. Additionally, it functions as an immunomodulator, lowers blood sugar levels, prevents blood clot formation, and exhibits antibacterial properties, among other health benefits. The Ganoderma species also contains a diverse range of 400 important bioactive chemical constituents, such as polysaccharides, triterpenoids, polysaccharide-peptide complex, β-glucans, lectins, natural germanium, adenosine, phenols, steroids, amino acids, lignin, vitamins, nucleotides, and nucleosides. These constituents possess distinct healing properties and are utilized to create various useful products derived from *Ganoderma* fruiting bodies, mycelia, and spores [7].

Figure 1: *Ganoderma lucidum* [8]**.**

The paragraph outlines the cytotoxic effects and proliferation rates on the HSSCC cell line influenced by different extracts including hot aqueous, cold aqueous, and ethanolic extract and different concentrations. This information is crucial to understand how the extracts might work in real-life applications and their potential as a therapeutic option.

Ganoderma lucidum extracts have been found to exhibit anti-cancer properties, including the ability to directly impede cell survival, inhibit cell growth by inducing cancer-specific cell cycle arrest through the suppression of cell cycle-related proteins, and trigger apoptosis [9].

The current pharmacological and clinical studies have shown that *G. lucidum* has anticancer action primarily by enhancing the immunological function of the host. Attaching to serum-specific proteins or leukocyte surfaces stimulates the activation of enhances the production of cytokines such as IL, interferon (IFN), TNF-a, nitric oxide (NO), and antibodies by the activated effector cells. Additionally, it has been shown to inhibit the development of cancer by controlling the response to DNA damage, the growth of cancer cells, and programmed cell death [10, 11].

Multiple prior research has investigated the possible anticancer properties of medicinal mushrooms. The research has concentrated chiefly on purifying, defining, and examining the bioactive chemicals present in the mushroom which are responsible for these effects. The fungus contains various chemicals, including polysaccharides and triterpenes, such as ganoderic acid, that are thought to be responsible for its anticancer activities [12].

Ganoderma lucidum comprises a diverse range of bioactive compounds, and its extract depends on the solvent used, such as water, which exhibits greater polarity than ethanol [13].

In cold aqueous extract, polysaccharides and in hot aqueous extract, flavonoids and phenolics have antioxidant activity, and ethanoic extract triterpenoids have anticancer activity [14, 15, 16]

This study aimed to compare the cytotoxic effects of three different types of crude extracts from *G. lucidum* (hot aqueous, cold aqueous, and ethanolic) on human skin squamous carcinoma cells to provide insights into potential anti-cancer properties of *G. lucidum* extracts especially the activity of bioactive compounds and their relevance to skin cancer treatment in the local Iraqi environment. This may enable development of more efficient and less toxic alternatives to synthetic chemotherapy agents.

2. Materials and method

2.1 Mushroom collection

Ganoderma lucidum (Reishi mushroom powder) were purchased from DXN Company (Malaysia).

2.2 Preparation of crude ethanolic extract

Ethanolic extract was prepared by dissolving 25 gm of *G. lucidum* fine powder in 250 ml of 80% ethanol at a ratio of 1:10 w/v between the powder and the solvent. The maceration process was conducted on a magnetic stirrer at room temperature for 48 hours. The flask was securely sealed using aluminum foil and parafilm during extraction. The extraction solution underwent centrifugation at 3000 rpm and filtration through the Whatman No. 1 filter paper. The resulting supernatant was then dispensed into glass Petri dishes and dried in an incubator at 40°C until complete desiccation. The dried extract was dissolved in a small quantity of DMSO solvent, and then the volume was completed to the mark with D.W. before utilization [17].

2.3 Preparation of crude aqueous extracts

In the case of hot aqueous extract, 25 gm of powdered sample was dissolved in 250 ml of sterilized distilled water at a ratio of 1:10 w/v on a hot plate at 80°C for the first 6 hours; then, the extraction continued for 48 hours without heating. Meanwhile, the cold aqueous extract was subjected to continuous agitated for period of 48 hours at room temperature. To prevent contamination and ensure airtight conditions, the flask was securely sealed using aluminum foil and parafilm during the maceration process. The aqueous phase underwent centrifugation at 3000 rpm, and the resulting supernatant was filtered, dried, dissolved in a proper solvent, and subsequently preserved at 4°C in a refrigerator [18, 19].

2.4 Preparation of dilutions

Two-fold dilutions were used to prepare a series of dilutions from a stock solution of ethanolic and aqueous extracts using RPMI medium without fetal calf serum provided by Sigma (USA) Company to produce a range of final concentrations of (7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000, 2000, and 4000) µg/ml.

2.5 Gas chromatography-mass spectrum analysis

The bioactive compounds in *G. lucidum* extracts (ethanolic, hot aqueous, and cold aqueous) were analyzed using an Agilent 6890 GC-mass system (Agilent, USA) connected to an Agilent 5973N MSD. The ion source temperature was set to be 200°C, and the injection volume was 1.0 µl with a split ratio of 50:1. The capillary column used was HP-5MS (30 m x 0.25 mm ID x 0.25 µm film) from Agilent J & W, USA. The oven temperature was initially set at 100°C and then increased at a rate of 10°C/min until it reached a final temperature of 275°C after 20 minutes. The transfer line temperature was set at 220°C. Helium gas was used

as the carrier at a constant 1 ml/min of flow rate. Data was acquired using the Agilent GC/MSD Chem-Station Version D.02.00 [20].

2.6 Cytotoxic effects of Ganoderma lucidum extracts on oral squamous carcinoma cell line

a. Cancer cell lines

1. The squamous carcinoma cell line of the oral was utilized at passage 27.
2. The cells were cultured using a tissue culture medium (RPMI) provided by Sigma (USA) Company, supplemented with 5% Fetal Calf Serum (FCS) provided by flow Laboratories (England).

b. Cancer cell lines culture

1. The experiment involved the placement of cancer cells within tissue culture flasks 25 cm2, which were maintained horizontally at a temperature of 37°C.
2. After the cells have achieved confluent monolayer, a subculture initiated by discarding the previous growth medium without serum and rinsing the cells with phosphate-buffered saline (PBS).
3. The cancer cells were harvested by adding Trypsin-versin' (T.V.) to the cell surface and allowing it to incubate for 10-15 minutes while gently agitating the cells.
4. The T.V. was removed, and the tubes were placed in an incubator at 37°C for 1-2 minutes. The tube was gently hit to facilitate the detachment of cells.
5. A volume of 20 ml of the culture medium supplemented with serum was introduced into the flask, followed by the even distribution of 10 ml of fresh confluent cells into new tissue culture flasks, thereby facilitating sub-culturing. The cells were subjected to incubation at a temperature of 37°C, and this procedure was repeated for subsequent subcultures or passages as required.
6. The cells were subsequently assessed for confluency to detect the number of viable cells in a slide chamber by employing a 1% trypan blue solution in a hemocytometer.

c. Cytotoxicity assay

Following the cell culturing process, the cytotoxic activities of *G. lucidum* extracts were assessed on cancer cell lines by the following steps [21]:

1. Harvesting: The cancer cells were collected by adding a trypsin-versene solution following the cell culturing process. 20 ml of culture medium with serum was introduced into the flask. The cells were subsequently assessed for confluency by employing a 1% trypan blue solution in a hemocytometer.
2. Cells seeding: A 96-well microtiter plate with a flat bottom was utilized for the experiment. In each well, 100 µl of tumor cells suspension was added. Cell numbers were adjusted into (10^4 cells/well) in a culture medium using a hemocytometer. The seeded plate was covered and incubated for 24 hours in a CO_2 incubator at 37°C, 5% CO_2, and 95% humidity to promote the adhesion of cells to the plate surface [22].
3. Exposure of cancer cells to the mushroom extracts: The following day, after seeding the cells, a series of half dilutions of each type of *G. lucidum* extract were simultaneously prepared in sterile tubes using a culture medium without serum, resulting in concentrations (7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000, 2000, and 4000) µg/ml. The plate cover was lifted, and the culture medium was removed. In column 1, the negative control was represented by adding 200 µl of culture medium without serum. In columns 2 to 12, 200 µl of the diluted extracts were added. Two columns were designated for each concentration. The plates were securely sealed and placed in an incubator at 37°C for 24 hours, 48 hours, and 72 hours. Each extract was administered in triplicate, using three distinct cell lines.

4. Cytotoxicity assay: After incubation, the mixture was carefully aspirated and rinsed with PBS three times. Subsequently, 28 µl of MTT stain with a 2 mg/ml concentration was added to each well. The plate was kept in the CO2 incubator for 2 hours. Afterward, the plate was rinsed with phosphate buffer saline (PBS) several times to ensure that the dye was removed. Once the plates were dry, 130 µl dimethyl sulfoxide (DMSO) was added to eliminate any remaining stain then the plates were placed in a microshaker for 15 minutes. The absorbance at a wavelength of 492 nm was recorded using a microplate spectrophotometer. The mean inhibitory rate (I.R) was measured according to the "Eq. (1)" [23], and the mean proliferation rate (PR) was calculated according to the "Eq. (2)" [24].

$$IR\ \% = (A - B)/A \times 100 \quad (1)$$

$$PR\ \% = B/A \times 100 \quad (2)$$

where $IR =$ inhibition rate; $PR =$ proliferation rate, $A =$ absorbence for negative control, $B =$ absorbence for test

2.7 Statistical analysis

The Statistical Analysis System: SAS (2018) program was employed to detect the effect of different factors in study parameters, including time, concentration, and type of extract. The least significant difference (LSD) test (Analysis of Variation-ANOVA) was used to compare the means in this study with probability p=5% significantly [25].

3. Results

3.1 GC- MS analysis

GC-MS chromatograms of the Reishi mushroom crude extracts exhibited different active compounds. *G. lucidum* is composed mainly of organic compounds such as alcohols, aldehydes, esters, and ketones. Some ketones are cyclohexanone, 3-hydroxy, cyclohexanone, 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, and ethanone, 1-(2-methylcyclopropyl)-. Alcohols are abundant in edible mushrooms and found in different forms, such as 2-furan methanol as furfural alcohol, 3-hexanol, cyclopentanol as a primary alcohol, and 2-methyl-2-nonanol as a fatty alcohol. The main volatile aroma compounds identified were aldehydes, such as 2-ethyl-trans-2-butenal.

The total sum of fatty acids was relatively high in *G. lucidum*. The primary fatty acid identified in G. lucidum extracts was palmitic acid, including forms like hexadecanoic acid, 2,3-dihydroxypropyl ester, and n-hexadecanoic acid. Other significant fatty acids included oleic acid and fatty acid esters such as 3-methylpentanoic acid and 2-hydroxy-, methyl ester. Additionally, organic compounds like pyridines were present, including 2-picoline and 6-nitro-.

Other organic compounds found in the form of Epoxides cyclic ether such as Oxirane, 3-butenyl-, in the form of Aldoxime such as Pentanal, oxime, in the form of polyether such as oxirane, ethoxymethyl-, in the form of nitrile such as Pentanenitrile, 4-methyl-, and flavonoid organic compound such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (Figure-2 a, b, c).

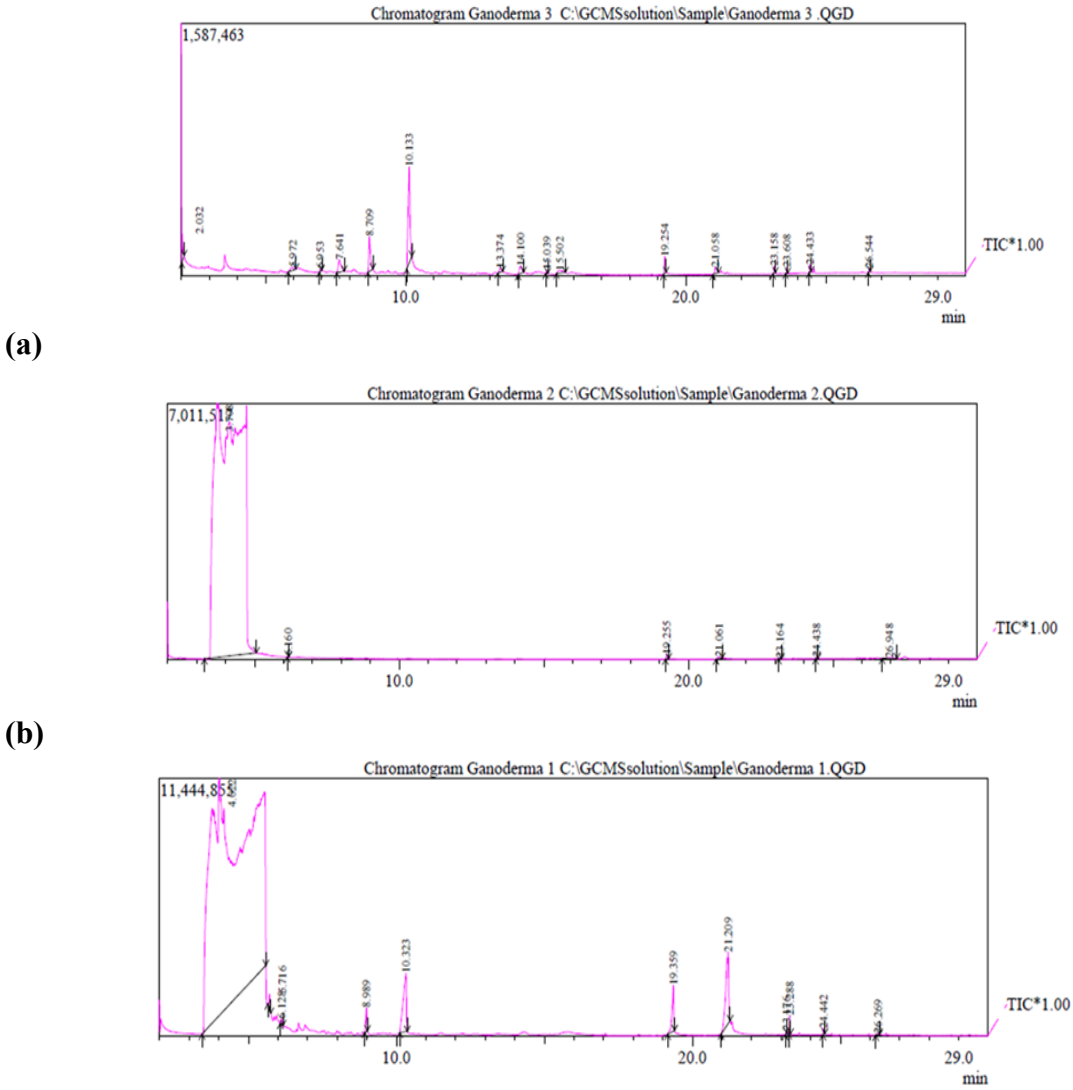

Figure 2: Gas chromatogram of *Ganoderma lucidum* extracts. (a): Cold aqueous extract, (b): Hot aqueous extract, (c): Ethanolic extract

3.2 Cytotoxic effect of Ganoderma lucidum extracts on (HSSCC) line

The results indicated that the cytotoxic effect of *G. lucidum* extracts (ethanolic, hot aqueous, cold aqueous) was based mainly on the type of the extract, its concentration, and exposure time. The cytotoxic effect of *G. lucidum* extracts on the HSSCC line revealed that the inhibition rate is influenced by the concentration, exposure time, and type of extract used. A high inhibition rate was observed at 4000 µg/ml concentration across all three extracts. The effect gradually diminished as the concentration decreased, while divisions and proliferation were stimulated. At a concentration of 4000 µg/ml, a significant inhibition rate ($P \leq 0.05$) was achieved: 77%, 83%, and 86% after 24, 48, and 72 hours respectively when treated with ethanolic extract; 62%, 66%, and 72% after 24, 48, and 72 hours when treated with hot aqueous extract; 48%, 57%, and 62% after 24, 48, and 72 hours when treated with cold aqueous extract compared to the control group, which had 100% cell viability.

The corresponding cell viability percentages were 23%, 17%, 14% after 24 hours, 38%, 34%, 28% after 48 hours, and 52%, 43%, and 38% after 72 hours after treatment with ethanolic, hot aqueous, and cold aqueous extract respectively (Figure 3 a, b, c).

At lower concentration of 1000 µg/ml, the percentages of viable cells were the ethanolic, hot aqueous, and cold aqueous extracts were 103%, 105, 108% after 24, 48, 72 hours; 102%, 103%, 110% after 24, 48, 72 hours; 103%, 103%, 110% after 24, 48, 72 hours respectively compared to the control (viability 100%) (Figure 3 a, b, c).

A statically significant proliferation rate (P≤ 0.05) was observed, reaching 126%, 126%, 132% after 24, 48, 72 hours, respectively, following treatment with ethanolic extract, 124%, 123%, 133% after 24, 48, 72 hours when treated with hot aqueous extract, 120%, 118%, 121% after 24, 48, 72 hours after treatment with cold aqueous extract at the concentration 62.5 µg/ml (Figure 3 a, b, c).

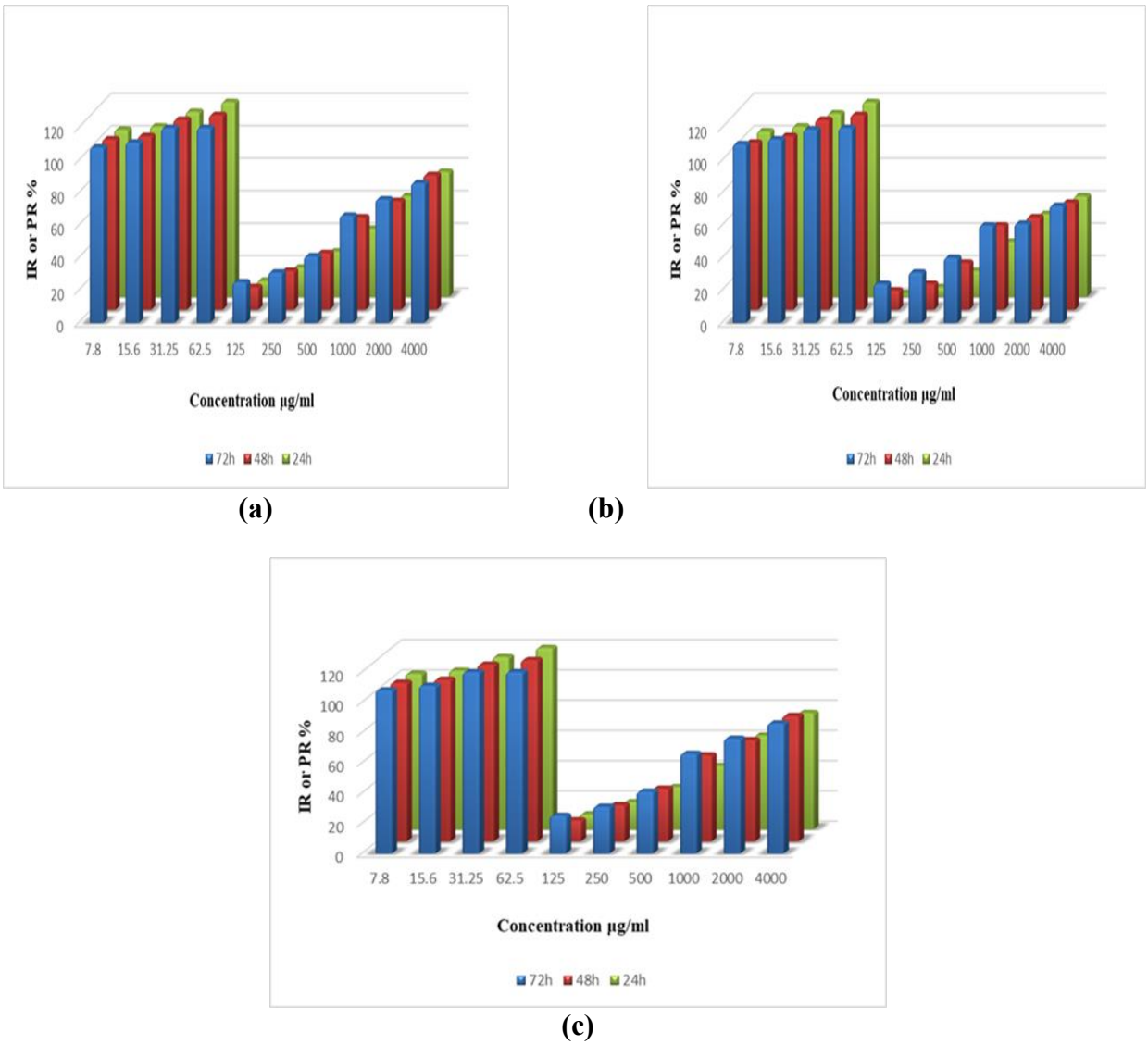

Figure 3: Cytotoxic effect of *Ganoderma lucidum* extracts (ethanolic extract, hot aqueous extract, and cold aqueous extract) on human skin squamous carcinoma cell line (HSSCC) after different exposure time, (a): Ethanolic extract, (b): Hot watery extract, (c): Cold watery extract. IR: Inhibition rate, PR: Proliferation Rate.

Upon comparing the viability between all treatments at corresponding times of exposure, it was found that, in the three periods of exposure (24, 48, and 72) hours, the significant growth inhibition (P≤ 0.05) started from a concentration of 125 µg/ml to a concentration of

4000 µg/ml with low cell viability. On the other hand, cell viability with a low inhibition rate at low concentration (7.8 µg/ml) of the three extracts reached significant cell proliferation ($P \leq 0.05$) at 62.5 µg/ml concentration. There is a direct proportion between the concentration and the inhibition rate (IR) and an inverse proportion between the concentration and cell viability (PR) (Figure 4 a, b, c).

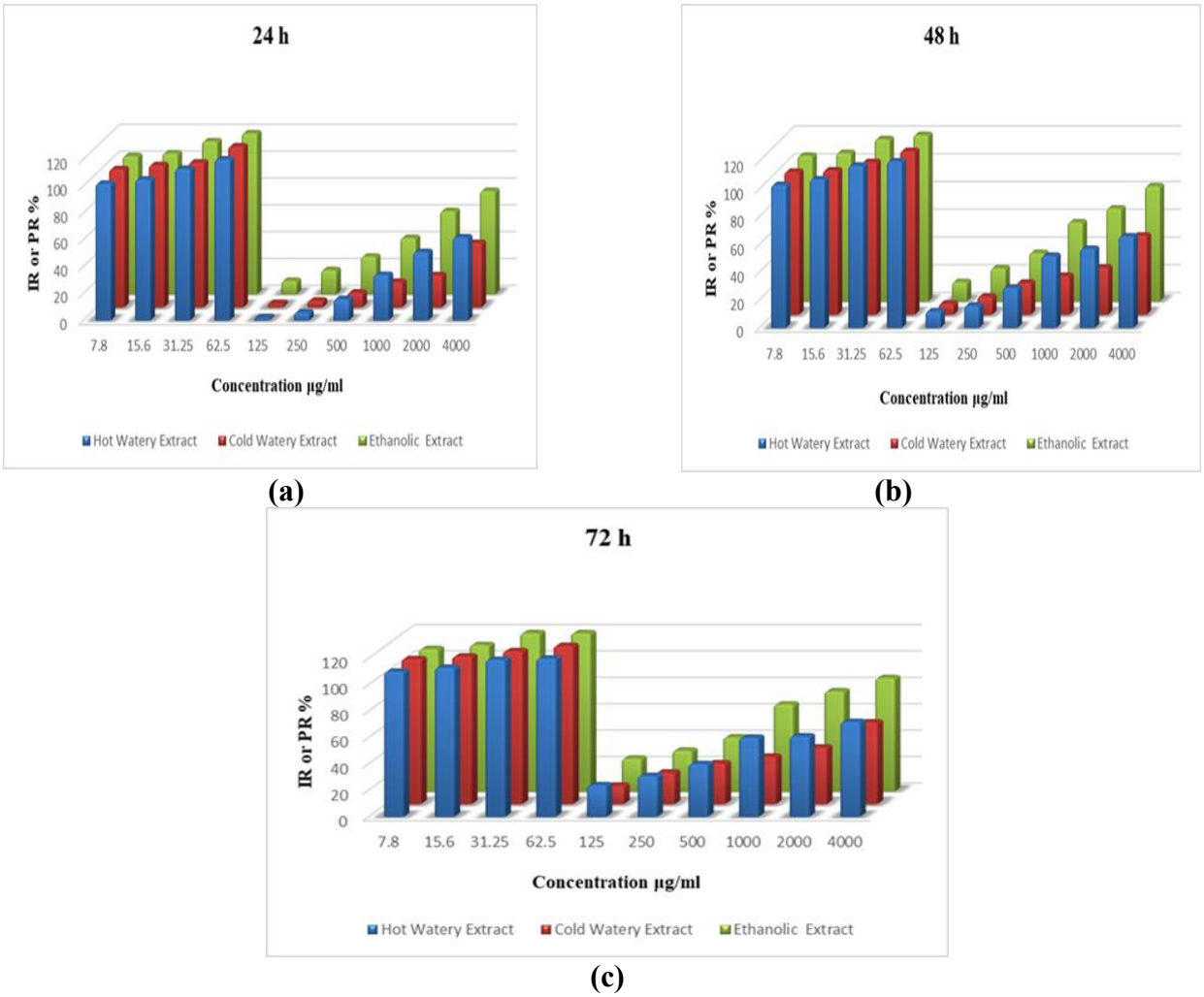

Figure 4: Comparison between *Ganoderma lucidum* extracts (ethanolic extract, hot aqueous extract, and cold aqueous extract) on human skin squamous carcinoma cell line (HSSCC) after different exposure time, (a): Three extracts after 24 hours, (b): Three extracts after 48 hours, (c): Three extracts after 72 hours. IR: Inhibition rate, PR: Proliferation Rate.

4. Discussion

The active compounds present in *G. lucidum* extracts exert an impact on the cancer cells through both direct and indirect mechanisms, with latter involving the immune system. Certain flavonoids have been found to have anticancer properties [3]. Palmitic acid is another compound reported to have anticancer activity [26]. In addition, some studies have shown that n-hexadecanoic acid can potentially inhibit the growth of HCT-116 cancer cells with an excellent IC50 value [27]. Furthermore, fatty acids are known to have the ability to regulate the immune response by acting directly on T cells [28]. The potential of different bioactive compounds derived from *G. lucidum* in cancer treatment and the importance of the extraction method in obtaining these compounds.

This study assesses the potential of *G. lucidum* to kill cancer cells, which could be used as a foundation for future cancer treatments, evaluates the cytotoxic effects of three different types of extracts (alcoholic, hot aqueous, and cold aqueous) on a human skin squamous carcinoma cell line (HSSCC). Testing multiple types of extracts allows for a comparison of their effectiveness in inhibiting the growth of cancer cells. The extracts are tested at three different exposure periods: 24 hours, 48 hours, and 72 hours, understanding the time-dependent effects of each extract on the cancer cells. A two-fold dilution series ranging from 7.8 to 4000 µg/ml is used for each type of extract. The extracts are tested across various concentrations to determine the minimum effective dose and the dose-dependent effects. The MTT assay is used to detect cytotoxicity. This assay measures lactate dehydrogenase enzyme activity, which is a cell viability indicator. When the MTT dye is reduced, it forms water-insoluble purple formazan crystals that precipitate in the cell cytoplasm. Dimethyl sulfoxide (DMSO) is added to solubilize the insoluble formazan crystals, releasing them from the cells. This solubilization is necessary to measure the absorbance of the formazan, which indicates the number of viable cells remaining. The absorbance of the solubilized formazan is measured at 492 nm. The optical density (OD) obtained from this measurement reflects the number of viable cells, where a lower OD indicates a higher extract cytotoxicity. The cytotoxicity evaluation is based on calculating the inhibition rate percentage compared to a control group. However, evaluating the cytotoxic effect depends on calculating the percentage of the inhibition rate compared with the control, and the proliferation rate is calculated using the same principle [23, 24].

The study results agreed with other results; the effect of the three extracts depends on three factors: type of extract, concentration, and finally, time that the cells are exposed to these extracts. The extracts decrease cell viability in a concentration-dependent manner; in other words, high concentrations inhibit cancer cell growth in a high percentage, meanwhile the inhibiting rate decreases with low concentrations [9].
Extracts derived from the Reishi mushroom have the ability to suppress the proliferation of various cancer cell types. However, it depends on concentration and time. It increases directly proportional to their increase [29].

In which aqueous and ethanolic extracts not inhibited cell proliferation after one day of treatment. In contrast, the exposure for three days resulted in a significant suppression of cell growth; more significant suppression was observed with the ethanolic extract, which is more inhibitory than the aqueous extract. At a concentration of 10 µg/ml ethanolic extract, approximately 50% inhibition of cell growth was found. Other studies including cell line and the period of treatment reported significant anti-proliferation differences *in vitro*; ethanol extract of *G. lucidum* elustrated a cytotoxic effect against HeLa (38.11–109.04) µg/ml and A549 (26.48–167.08) µg/ml cell lines. However, this cell lines were cultured with the indicated concentrations of ethanol extract for 72 hours [16].
Significant differences exist in the cytotoxicity levels of different extracts when applied to a specific cell line. The period during which the cancer cells are exposed to the extracts affects the anti-proliferative outcomes significantly. The study reported that low concentrations of the extracts show minimal inhibitory effects on the cancer cells. The effectiveness of the extract appears to be concentration-dependent, with higher concentrations potentially exhibiting enhanced inhibition of cell proliferation. There is a noted increase in cancer cell proliferation, specifically after 24 hours of exposure to the extracts (low inhibition rate) compared with the control (100%). It suggests that the cells may initially react to the extracts

with increased growth, possibly due to a stimulatory effect or inadequate dosage to induce cell death.

The extracts demonstrated a considerable difference in their cytotoxic effects on the cell line, and the length of exposure played a crucial role in the significant anti-proliferative outcomes seen *in vitro*.

Numerous studies showed that there is a significant cell inhibition of human breast cancer MCF-7 cell lines at higher concentrations. The cytotoxicity effect was observed after 24 hours of incubation. Cell inhibition was increased in a concentration-dependent manner. Among the extracts, the aqueous extract produced 71% cell inhibition, and 29% of viable cells were observed. Similarly, 36% of viable cells were noted in ethanol extracts at 1000 µg/ml concentrations [30].

Reishi mushroom has been shown to have cytotoxic effects on several tumor cells, such as Rat adrenal pheochromocytoma cells (PC12), mouse melanoma B16 cells, human cervical carcinoma cells (Hela), and human lung carcinoma cells. After 48-hour of treatment, cell viability significantly declined as the concentrations increased. The ethanolic extract of *G. lucidum* had cytotoxicity effects on the viability of cervical carcinoma, lung cancer, and colon cancer. The IC50 value is the concentration required to inhibit 50% of the cancer cells and ranges from 120 to 500 µg/ml [31].

Biocomponents found in *G. lucidum* are effective in treating different types of cancer. The potential ways Reishi mushroom bio-compounds could be used in cancer treatment. Ahmad's considerations include activating the immune response in the host cell, inducing cell differentiation, inhibiting angiogenesis, directly attacking tumor cells, inhibiting urokinase-type plasminogen activator and receptor expression in cancer cells, and inhibiting phase II metabolizing enzymes [9, 32].

Various natural sources, such as *Vinca rosea* and the *Yucca* plant, have traditionally been used to treat cancer cells, including those in the human brain and cervix [33] it is advised that further research explore *G. lucidum* effects on brain cancer and cervical cancer. In addition to the assessment of Reishi mushroom (provided by the DXN company), further studies are recommended to evaluate the effects of *G. lucidum* on cancer cells in vivo [34]. Additionally, research should explore the potential synergistic effects of *G. lucidum* with gold nanoparticles to enhance its efficacy [35]. Finally, it is also essential to study its impact on the autophagy process, investigate the cytotoxicity of *G. lucidum* extracts on myeloblasts isolated from AML patients' blood, and the genotoxicity of *G. lucidum* extracts on bone marrow cells [36].

Conclusion

In conclusion, the study revealed that ethanolic and aqueous extracts of *G. lucidum* contain bioactive compounds with significant anti-cancer properties. Ethanolic extract have a better impact on cancer cells than aqueous extracts due to the more active compounds in ethanolic extracts. Concurrently, the extract exhibits a multifaceted profile of bioactivity, encompassing anticancer, antioxidant, and immunomodulatory activity. In particular, the extracts had a dual impact on skin cancer cells. At elevated concentrations, they inhibited the growth of cancer cells, suggesting potential cytotoxic effects that could be harnessed for therapeutic applications. They stimulated cell proliferation at low concentrations, suggesting a hormesis effect; this means that low doses of the extract may trigger adaptive cellular responses that promote growth. Further research may be needed, including investigating the molecular mechanisms underlying the hormesis effect observed with low concentrations of *G. lucidum* extracts. Identifying the therapeutic window for *G. lucidum* extracts, where the

anti-cancer effects are maximized while minimizing potential proliferative side effects at lower doses.

5. Conflict of interest

The authors have no conflicts of interest to declare.

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No grant or financial support was received from any governmental or private sector for this study.

7. Author's contribution

Study conception and design, Acquisition of data analysis, drafting of manuscript: (Hala M. Mahmud). Interpretation of data, critical revision: (Hind H. Obaid).

8. Ethical responsibilities of authors

This study did not involve any tests on humans or animals. The authors have no ethical conflicts to declare (Ref. CSEC/1221/0082).

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