

# Study of the Cytotoxic Activity of Alcoholic and Aqueous Extracts of Iraqi Aromatic Rice Bran on B16 Cell Line

Abeer Saeed Abdualhadi<sup>1\*</sup>

<sup>1</sup> Department of Quality Control, Grain Trade, Ministry of Commerce, 10001 Baghdad, IRAQ.

\*Contact: [abeersaeed772021@gmail.com](mailto:abeersaeed772021@gmail.com)

## Article Info

Received  
21/04/2023

Revised  
02/06/2023

Accepted  
06/07/2023

Published  
30/12/2023

## ABSTRACT

The test was conducted on a sample consisting of mixing three samples of Iraqi Aromatic rice (Amber) bran obtained from the private mills in Najaf Governorate. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method was used to study the cytotoxic effect of alcoholic and aqueous extract of Iraqi Aromatic rice bran on B16 cell line, it is a murine tumor cell line used as a model to study human melanoma. The results showed that all concentrations of alcoholic extract (5, 10, 20, 40, and 80) % caused a significant inhibition on the B16 cell line. The inhibition rate reached to (13.00, 27.00, 47.33, 71.67 and 86.67) % respectively, after 48 hours while the cell viability was (87.00, 73.00, 52.67, 28.33 and 13.33) % with a statistically significant difference at  $P \leq 0.05$  compared to the control. The results showed that the aqueous extract had less inhibition effectiveness (for the same concentrations) after 48 hours, (10.00, 19.33, 27.00, 46.67 and 66.33) % and the cell viability rate was (90.00, 80.67, 73.00, 53.33 and 33.67) % respectively. The study shows that both alcoholic and aqueous extract prepared from Iraqi Aromatic rice bran had a toxic and anti-proliferative effect on B16 cell line, and this effect increases with increasing concentration. Treatment of these cells with the two extracts caused shrinkage, a decrease in numbers, and a clear morphological change in this type of cells.

**KEYWORDS:** Rice bran, anticancer, B16 cell line, botanical studies.

## الخلاصة

تم إجراء الاختبار على عينة مكونة من خلط ثلاث عينات من سحاله الأرز العراقي العطري (العنبر) تم الحصول عليها من المجارش الاهلية في محافظة النجف الاشرف. تم استخدام طريقة MTT لدراسة التأثير السام للخلايا للمستخلص الكحولي والمائي لسحاله الأرز العراقي على خط خلايا B16، يستخدم خط خلايا ورم الفئران كنموذج لدراسة سرطان الجلد البشري. أظهرت النتائج أن جميع تراكيز المستخلص الكحولي (5، 10، 20، 40، 80) % تسببت في تثبيط معنوي لخط الخلية B16. بلغ معدل التثبيط (13.00، 27.00، 47.33، 71.67، 86.67) % على التوالي بعد 48 ساعة. بينما كانت حيوية الخلايا (87.00، 73.00، 52.67، 28.33، 13.33) % مع وجود فرق ذي دلالة إحصائية عند  $P \leq 0.05$  مقارنة بالسيطرة. أظهرت النتائج أن المستخلص المائي كان له فعالية أقل في التثبيط (لنفس التركيزات) فقد وصل بعد 48 ساعة (10.00، 19.33، 27.00، 46.67، 66.33) % ومعدل حيوية الخلايا (90.00، 80.67، 73.00، 53.33، 33.67) % على التوالي. أظهرت الدراسة أن كلا المستخلصين الكحولي والمائي المحضر من سحاله الأرز العراقي العطري له تأثير سام ومضاد للتكاثر على خط الخلايا B16، ويزداد هذا التأثير مع زيادة التركيز. تسبب علاج هذه الخلايا بالمستخلصين في انكماش ونقص في الأعداد وتغير شكلي واضح في هذا النوع من الخلايا.

## INTRODUCTION

According to the World Health Organization (WHO), cancer is one of the leading causes of death worldwide and caused about 10 million deaths in 2020. Among them (1.20 million) cases are due to skin cancer. Skin cancer is one of the most common malignant diseases. Cancer occupies non-melanoma skin in New Zealand ranks first for both women and men in terms of incidence, while melanoma skin cancer ranks third for both

genders [1]. Some genetic and environmental factors contribute to its spread and its development such as exposure to ultraviolet radiation, practicing some professions such as tanning, ionization and radiotherapy, exposure to chemicals and inhalation of fumes in enclosed spaces, dealing with arsenic and polycyclic aromatic hydrocarbons, chronic dermatitis, immunosuppression, personal and family history [2]. Thus, many researchers seek in their studies to find effective treatments or

alternatives to treat cancer. These medicines must be made from natural materials that have no side effects, available and cheap. Rice bran is the hard outer layer that is obtained during the manufacture of rice grains (to turn the grains into the desired white shape). Rice bran usually consists of a mixture of aleurone and husk. It is rich in fatty acids and contains proteins, dietary minerals and vitamins [3]. The healthy oil is approximately 12-18.5% of rice bran (47% monounsaturated, 33% polyunsaturated), and 20% saturated fat in addition to other ingredients, including tocotrienols, gamma-oryzanol, and beta-sitosterol [4]. As for the bran content of proteins, it reaches 10-18% of the bran and includes many types: globulin (soluble in salt), albumin (soluble in water), prolamin (soluble in alcohol) and glutelin (soluble in alkali). Rice bran protein contains all the essential amino acids, unlike rice protein itself [5][6]. Most researchers strive to isolate the biologically active components of rice bran and reveal their functions. Rice bran contains bioactive compounds produced by the plant as secondary compounds. Most of them are bound to cell walls and have biological activities such as antioxidant, antimicrobial, anticancer, antidiabetic and hypolipidemic, including phenolic (ferulic acid, vanillic acid, p-coumaric acid, iso ferulic acid, syringic acid and sinapic acid), flavonoid (quercetin-3-glucuronide, rutin and myricetin), tocopherols ( $\alpha$ -tocotrienols,  $\gamma$ -tocopherols,  $\alpha$ -tocopherols,  $\delta$ -tocopherols,  $\gamma$ -tocotrienols, and  $\delta$ -tocotrienols),  $\gamma$ -oryzanol (24-methylene cycloartenyl ferulate, cycloartenyl ferulate,  $\beta$ -sitosteryl ferulate and 2 campesteryl ferulate), phytosterols ( $\beta$ -sitosterol, stigmasterol, and campesterol) and squalene [7]. The mechanism of action for the active compounds extracted from rice bran is to be involved in the process of regulating and stimulating the process of apoptosis of cancer cells. For example, phytic acid stimulates apoptosis of liver cancer cells and has a proactive effect on the colon cancer cell line HT-29 by regulating the expression of proteins. Cycloartenyl ferulate is one of the phenolic compounds from rice bran, was also found to play a role in activating death receptors and triggering apoptosis in colon cancer cells. Tocotrienols present in rice bran are involved in mediating apoptosis in cancer cells, such as gamma-tocotrienol that induces apoptosis in gastric adenocarcinoma. In addition, delta-tocotrienol induces apoptosis in human colorectals. Rice bran extracts and FRB are potential chemo-preventive

agents and enhance the susceptibility of cancer cells to chemotherapy drugs [6]. Kim *et al.* [8] studied the effect of  $\gamma$ -oryzanol oil extracted from rice bran on mice with colon cancer. He found that the tumor mass was significantly lower in the mice that took  $\gamma$ -oryzanol. This is due to the following biological reasons: An increase in the cytolytic activity of splenic natural killer (NK) cells; increases in the release of tumor necrosis factor pro-inflammatory cytokines from macrophages; a significant decrease in mRNA and protein expression genes, causing inhibition of the formation of new blood vessels within the tumor, this was confirmed by the ELISA test for cancer cells, where gene expression decreased by up to 30%. Most of the studies examined the effect of active substances in rice bran on different cancer lines, but did not take into account their effectiveness on skin cancer cells. There were few studies in this aspect. This study focused on using a special type of rice bran, which is the Iraqi Amber plant to study its effect on skin cancer.

## MATERIALS AND METHODS

### Sample Preparation

The final assay sample was obtained by mixing three rice bran samples of equal weights. The samples were taken from different regions in Al-Najaf Governorate. The three samples were examined first to ensure that they are free of any damage, then they were purified from impurities, and finally they were mixed to obtain a sample representative of the quantity.

### Preparation of the Alcoholic Extract

The alcoholic extract of rice bran was prepared using the same method as that of [9] with some modification. 20 g of bran powder was weighed and mixed with 200 ml of ethanol. The mixture was placed in a sterile 500 mL conical glass flask. The vial was closed tightly with a sterile plastic cap. It was transferred to a shaking incubator at 200 rpm for 24 hours. After the end of the period, the extract was filtered using filter paper. The filter is then placed on a vibrating heater until it evaporates.

### Prepare the Aqueous Extract

The same method followed in preparing the alcoholic extract with the replacement of ethanol with distilled water.

### B16 Melanoma Cell Line

Melanoma B16 is a murine tumor cell line used for research as a model of human skin cancers. It is a

unique model for studying the formation of malignant tumors in the skin, and it was one of the first cells used for research studies. In this study the cancer cell line belonged to mouse strain C57BL/6, and the passage number was 28. The B16 cell line (Sigma-USA) was obtained from the Iraq Biotech Cell Bank Unit.

### Preparation and Maintenance of B16 Cultures

B16 was routinely grown under standard conditions in 75 ml falcon dishes and incubated at 37 °C. The medium used for the growth of B16 cells was RPMI-1640. The medium was prepared using (10% Fetal bovine serum, 100 units/mL streptomycin, and 100 µg/mL penicillin). According to the method of [10][11] it was as follows: -

1. The B16 cell line was monitored for a confluent monolayer.
2. Cells were washed under sterile conditions with PBS, after the culture medium was withdrawn and discarded.
3. Trypsin/EDTA (0.025%) was added in an amount of (3 ml) to the culture, the flask should be carefully inverted to completely cover the monolayer, and incubated at (37 °C for 5 minutes). This solution was used for the purpose of lysing the cells and separating them from the falcon dishes.
4. RPMI-1640 culture medium (15 ml) was added and the plate was incubated at 37 °C with 5% CO<sub>2</sub>.
5. The process was repeated twice a week until it achieved 80% density.

### Prepare Cells for Toxicology Tests and Live Cell Counting

The method was carried out according to [12] using trypan blue dye technique. Dead cells absorb the dye while living cells remain transparent. It was according to the following steps:

Trypan blue stain solution (4%) was mixed with an equal volume of cell suspension. Then, an amount (10-20 µL) of the suspension was taken and transferred to the edge of (hemocytometer chamber) and the slide was left for (2 min.), The number of cells per square was counted using a light microscope with a 40X lens.

Before performing the test on a B16 cell line, its viability must be measured. The total number of cells and the concentration of live and dead cells

per ml were also calculated, in a manner [13][14]:  
-  
Equation (1) is used to calculate the percentage of viable cells:

$$C_{bt} (\%) = \frac{\text{number of unstained cells}}{\text{total number of cells}} \times 100 \quad (1)$$

Where  $C_{bt}$  (%) stands for Cell viability before testing, B16 Cells were re-cultured with Trypsin/EDTA twice a week, until 80% was achieved. to be valid for toxicity testing.

### Cytotoxicity Assay of Alcoholic and Aqueous Extract of Iraqi Aromatic Rice (Amber) Bran on B16 Cell Line

Different concentrations of alcoholic extract of Amber rice were prepared and tested for their cytotoxic effect according to the method [15][16] using 96-well plates, by MTT technique (3- 4, 5-dimethyl-2- thiazolyl-2, 5-diphenyl-tetrazolium bromide)

Note: The same method was used to test the aqueous extract of amber rice bran on B16 cell line.

1. The culture medium was used to prepare different concentrations (5, 10, 20, 40, and 80)%.
2. The old culture medium was discarded, for the cell line, 3 mL of Trypsin/EDTA was added in a 75 mL flask (to prepare the cell suspension) and incubated at 37°C with 5% CO<sub>2</sub> for 5 min.
3. Fifteen ml of growth medium supplemented with 10% fetal calf serum was added to form the cell suspension.
4. To achieve a confluent monolayer of B16 cells, 200 µL of cell suspension at a concentration (1 x 10<sup>4</sup> cells/mL) was placed into each well. The plate was closed and incubated at 37 °C with 5% CO for 24 h.
5. Culture medium was removed and 200 µL of different extract concentrations added to each well (3 replicates for each concentration).
6. Wells of control (B16 cell line without extract treatment) (three replicates for each concentration) were treated with 200 µl of culture medium and the plate was closed and incubated at 37 °C with 5% CO for 48 h.
7. After the end of the incubation period, the plate was washed with PBS after removing the extract and culture medium (to remove dissociated non-living cells).

8. MTT dye was prepared at a concentration of (2 mg/ml) and 28  $\mu$ l of this dye was added to each hole and incubated for 2.5 h. at 37 °C with 5% CO.
9. MTT dye was carefully removed and 130  $\mu$ L of DMSO (Dimethyl Sulphoxide) at 100% concentration was added to each well to dissolve crystals remaining in the well. Then, the plate was left at 37 °C for 15 min on a shaking incubator [17].
10. The absorbance was determined on a microplate reader at the wavelength at which the highest absorption occurs, which is 492 nm. Cell growth inhibition rate (percentage cytotoxicity) was calculated according to Equation (2) [18][19].

$$\text{cytotoxicity} = A - \frac{B}{A} \times 100 \quad (2)$$

A = optical density (of control)

B = optical density (of samples) [20].

11. The percentage of surviving cells or the vitality of cells (after testing) can be calculated using Equation (3):

$$\text{vitality rate} = 100 - R \quad (3)$$

R= rate of inactivation or cytotoxicity.

### Microscopic Examination of the B16 Cancer Cell Line

To complete the study of the effect of the alcoholic extract of rice bran and to visualize the shape of B16 cells under an inverted microscope, and note the morphological changes, the following steps were performed: A 24-well microtitration plates was taken and 200  $\mu$ l of cell suspension at a density of  $1 \times 10^5$  cell /mL injected into each well, then incubated with 5% CO at 37 °C. After 24 h. Following this step, culture medium was removed and 200  $\mu$ l of alcoholic extract was added to each well (for the control well, culture medium was added in the same quantity). The plate was sealed and incubated at 37° C with 5% CO<sub>2</sub> for 24 h. The plate was washed with distilled water and 20  $\mu$ l of crystal violet stain was added to each well and incubated at 37 °C for 15 min [21]. The excess of the dye was carefully removed with tap water. The cells were then examined under an inverted microscope at 100X magnification and images were taken with a digital microscope camera [22-24].

## RESULTS AND DISCUSSION

### Cytotoxic Activity of Alcoholic and Aqueous Extract on B16 Cell Line

To evaluate the metabolic activity of the cell, the MTT colorimetric assay method was used. The anti-proliferative activity of the alcoholic and aqueous extract of Iraqi rice bran (amber) was tested by examining its ability to inhibit the proliferation of B16 cells, which are mouse melanoma cells approved by researchers to study human skin cancer. Alcoholic and aqueous extract was prepared at different concentrations. Each concentration was tested in three replicates on a cancer cell line in order to verify the inhibitory effect. The results were recorded for the effect of both extracts (Table 1 and Table 2) and a comparison was made between them, as well as a comparison was made between the results of different concentrations of the substance under examination, before and after treatment, and the t-test was used to analyze the data.

**Table 1.** Cytotoxic effect and cell viability of different concentrations of alcoholic extract of amber rice bran on B16 cell line after 48 h. of exposure.

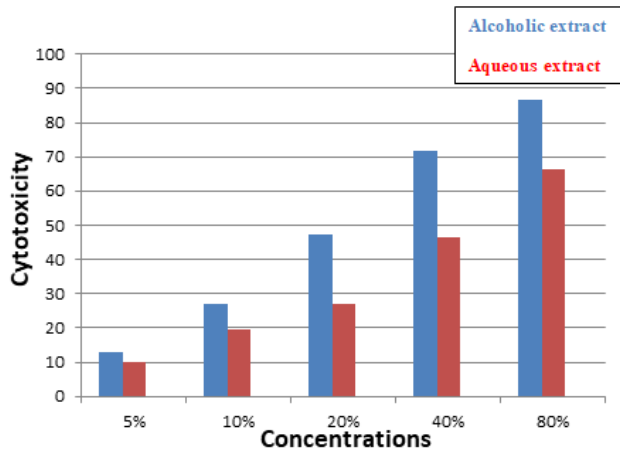
Concentrations of alcoholic extract	Cytotoxic effect% $\pm$ SD	Viability of cell%
control	0%	100%
5%	13.00 $\pm$ 2.30e	87.00
10%	27.00 $\pm$ 2.51d	73.00
20%	47.33 $\pm$ 2.84c	52.67
40%	71.67 $\pm$ 3.28b	28.33
80%	86.67 $\pm$ 1.85a	13.33

**Table 2.** Cytotoxic effect and cell viability of different concentrations of aqueous extract of amber rice bran on B16 cell line after 48 h. of exposure.

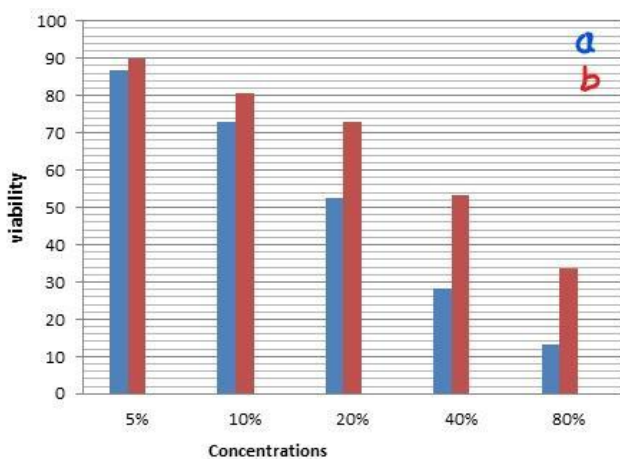
Concentrations of aqueous extract	Cytotoxic effect% $\pm$ SD	Viability of cell%
control	0%	100%
5%	10.00 $\pm$ 1.20e	90.00
10%	19.33 $\pm$ 2.11d	80.67
20%	27.00 $\pm$ 2.32c	73.00
40%	46.67 $\pm$ 1.83b	53.33
80%	66.33 $\pm$ 1.06a	33.67

The results showed that the alcoholic and aqueous extracts (Figure 1) of the tested rice bran had a cytotoxic effect against B16 cells line, as shown in the first and second tables, respectively. The efficacy is highly dependent on the type of extract and its concentration, increasing the concentration is inversely proportional to the viability of B16 cells. In addition, all treatments caused a significant inhibition rate with a statistically significant

difference at  $P \leq 0.05$  compared to the control (Figure 2). This is consistent with the study carried out by [7] where they indicated that rice bran extract contains active substances (phenolic content,  $\gamma$ -tocotrieno, Peptides and polysaccharides) had antitumor activity and anti-cancer cell anti-proliferation efficacy, and had effect against colon and rectal cancer lung, liver, and breast cancer cells. It also agrees with what [25] indicated that the alcoholic extract is more effective than the aqueous extract of Iraqi amber rice bran on cancer cell lines.



**Figure 1.** Cytotoxic effect of alcoholic and aqueous extract of the tested rice bran against B16 cells line



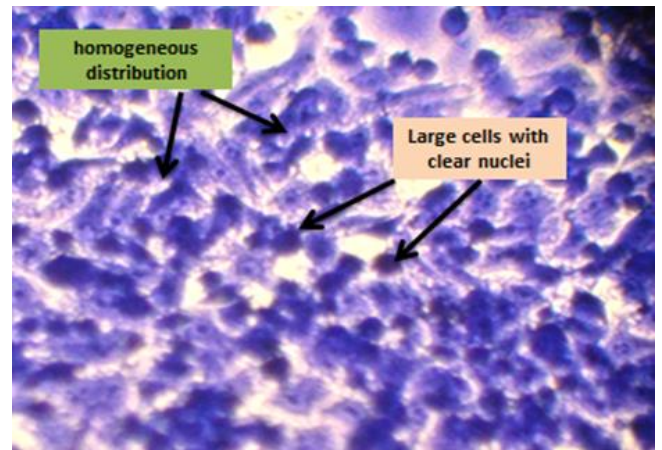
**Figure 2.** Cell viability% effect of alcoholic and aqueous extracts of the tested rice bran against B16 cells line. a) Alcoholic extract. b) Aqueous extract.

### Microscopic Examination

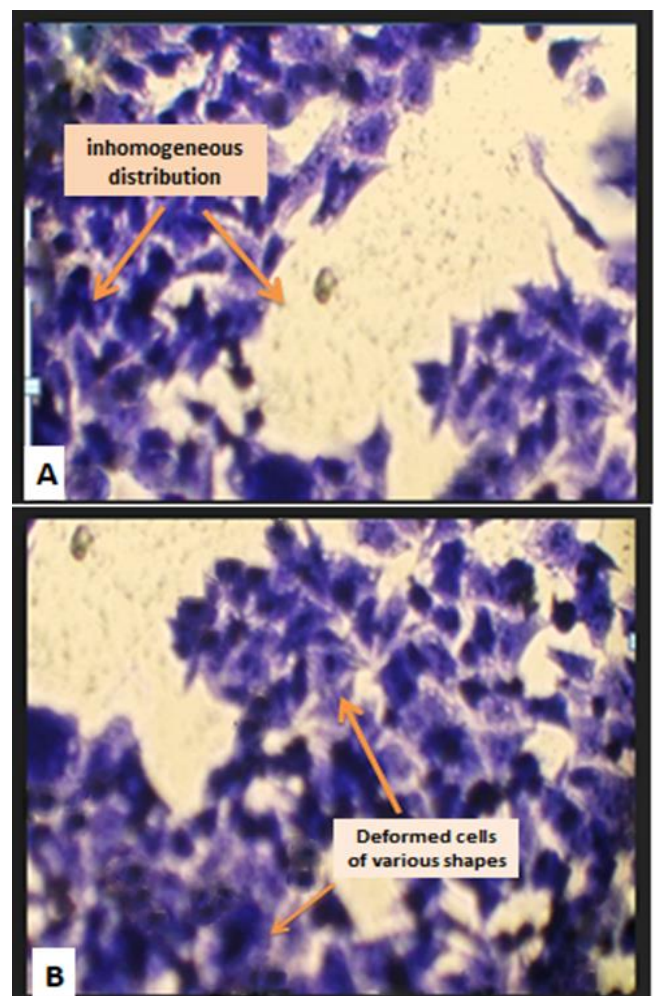
When examining the cells under an inverted microscope before treatment, it was noticed that there was an abundance of cancer cells with bulging and clear nuclei. The shape was consistent and regular with agglutination (see Figure 3).

While examining the cells after treatment, a decrease in numbers, voids and irregular

distribution, deformation and variation in cell shapes and sizes were observed as shown in (Figure 4).



**Figure 3.** Microscopic examination of the B16 cancer cell line (before) treatment under an inverted microscope with a 100X magnification lens.



**Figure 4.** Microscopic examination of the B16 cancer cell line (after) treatment under an inverted microscope with a 100X lens. a) Alcoholic extract. b) Aqueous extract.

We conclude from the results that the B16 cell line is very sensitive to the alcoholic and aqueous extract of the Iraqi ambergris rice bran at these concentrations. This study is consistent with many other studies, as it indicated the presence of the effectiveness of rice bran extract against the development of cancer cells by studying its effect on types of cancer lines. [26] indicated that alcoholic extracts of rice bran were rich in flavonoids such as apiiin, and those compounds showed broad activity against Hep2, HeLa and MCF-7 cancer cell lines through its activity in inducing apoptotic. In addition, phenolic compounds have an important role in preventing the proliferation of cancer cells. By inhibiting vascular endothelial growth factor, which plays a key role in the growth and development of cancerous tumors [27] Furthermore, a rutin has been reported to induce apoptosis in cervical cancer cells through cell cycle arrest at G0/G1 phase [28]. Rice bran extract is rich in antioxidant compounds such as Saponarin, which is an important natural flavonoid and has a strong antioxidant effect, which is also found in rice bran extract. This may be among the mechanisms by which bran extract fights most types of cancer cells. Tocotrienols and tocopherols contribute to reducing the risk of cancer, especially if applied to the skin where it is quickly absorbed. It has properties in eliminating free radicals, inducing programmed cell death, and suppressing cell proliferation. It disrupts cell cycle progression and prevents tissue damage by scavenging and blocking free radicals. It has also been shown to increase the chemoprevention of cancer by stimulating anti-cancer immune responses and altering the tumor microenvironment [29].

## CONCLUSIONS

Rice bran can be used in the pharmaceutical industry because it contains effective compounds instead of being destroyed. Rice bran is an important cheap source for the production of medicines and an adjunct that can be used during the treatment of human skin cancer. We suggest increasing and intensifying scientific research to discover other properties of this substance. Precious. In this study, the ethanol extract of Iraqi amber rice bran was more effective than the aqueous extract, because it was rich in flavonoids, such as Apiiin and Rutin, both of which are known for their antioxidant effectiveness [25]. Flavonoids have been shown to have an important effect in

fighting cancer cell proliferation by reducing inflammation and eliminating on free radicals, as well as inhibiting the growth of cancer cells by stimulating apoptosis, suppressing the cell cycle, inhibiting angiogenesis and some important enzymes in growth tumor and its development [30]. The efficiency of the aqueous extract can be increased by changing the method of preparation or increasing the time. More studies using other carcinoma cell lines are needed to understand the exact mechanisms of action of Iraqi amber rice bran.

## ACKNOWLEDGMENT

The author is grateful to the members and management of Al-Mustansiriyah Journal of Science for cooperation and support in publishing this scientific article.

**Ethical Approval:** No ethical approval is needed.

**Disclosure and Conflict of Interest:** The authors declare that they have no conflicts of interest.

## REFERENCES

- [1] J. Ferlay, M.Colombet, I. Soerjomataram, D. M. Parkin, M. Piñeros, A. Znaor, & F.Bray. Cancer statistics for the year 2020: An overview. *International journal of cancer*, 2021,149(4), 778-789.  
<https://doi.org/10.1002/ijc.33588>
- [2] A.Hanlon. *Practical Guide to Skin Cancer*. Springer international Publishing,2018  
<https://doi.org/10.1007/978-3-319-74903-7>
- [3] H. Y. Shin, S. M. Kim, J. H. Lee, & S. T. Lim. Solid-state fermentation of black rice bran with *Aspergillus awamori* and *Aspergillus oryzae*: Effects on phenolic acid composition and antioxidant activity of bran extracts. *Food chemistry*,2019, 272, 235-241.  
<https://doi.org/10.1016/j.foodchem.2018.07.174>
- [4] S.Bhosale &D.Vijayalakshmi.Processing and nutritional composition of rice bran. *Current Research in Nutrition and Food Science Journal*,2015, 3(1), 74-80.  
<https://doi.org/10.12944/CRNFSJ.3.1.08>
- [5] T. Laokuldilok, & Y. Rattanathanan. Protease treatment for the stabilization of rice bran: Effects on lipase activity, antioxidants, and lipid stability. *Cereal Chemistry*,2014,91(6), 560-565.  
<https://doi.org/10.1094/CCHEM-02-14-0022-R>
- [6] Y. Yu, J. Zhang, J. Wang, & B. Sun. The anti-cancer activity and potential clinical application of rice bran extracts and fermentation products. *RSC advances*, 2019,9(31), 18060-18069.  
<https://doi.org/10.1039/C9RA02439E>
- [7] R. Andriani, T. Subroto, S. Ishmayana, & D. Kurnia. Enhancement Methods of Antioxidant Capacity in Rice Bran: A Review. *Foods*,2022, 11(19), 2994.

- <https://doi.org/10.3390/foods11192994>
- [8] S. P. Kim, M. Y. Kang, S. H. Nam, & M. Friedman. Dietary rice bran component  $\gamma$ -oryzanol inhibits tumor growth in tumor-bearing mice. *Molecular nutrition & food research*, 2012, 56(6), 935-944.  
<https://doi.org/10.1002/mnfr.201200057>
- [9] A. J. Al-Manhel, & A. K. Niamah. Effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes and causing spoilage of food. *Pakistan Journal of Food Sciences*, 2015, 25(3), 104-109.
- [10] A. G. Al-Ziaydi, A. M. Al-Shammari, M. I. Hamzah, & M. S. Jabir. Hexokinase inhibition using D-Mannoheptulose enhances oncolytic newcastle disease virus-mediated killing of breast cancer cells. *Cancer Cell International*, 2020, 20(1), 1-10  
<https://doi.org/10.1186/s12935-020-01514-2>
- [11] A. G. Al-Ziaydi, M. I. Hamzah, A. M. Al-Shammari, H. S. Kadhim, & M. S. Jabir. (December). The anti-proliferative activity of D-mannoheptulose against breast cancer cell line through glycolysis inhibition. In *AIP Conference Proceedings*, 2020, Vol. 2307, No. 1, p. 020023.  
<https://doi.org/10.1063/5.0032958>
- [12] D. C. Darling, & S. J. Morgan. *Animal cells: culture and media*. John Wiley & Sons, 1994.
- [13] K. S. Khashan, G. M. Sulaiman, S. A. Hussain, T. R. Marzoog, & M. S. Jabir. Synthesis, Characterization and Evaluation of Anti-bacterial, Anti-parasitic and Anti-cancer Activities of Aluminum-Doped Zinc Oxide Nanoparticles. *Journal of Inorganic and Organometallic Polymers and Materials*, 2020, 1-17  
<https://doi.org/10.1007/s10904-020-01522-9>
- [14] M. Jabir, U. I. Sahib, Z. Taqi, A. Taha, G. Sulaiman, S. Albukhaty, & H. Rizwana. Linalool-Loaded Glutathione-Modified Gold Nanoparticles Conjugated with CALNN Peptide as Apoptosis Inducer and NF- $\kappa$ B Translocation Inhibitor in SKOV-3 Cell Line. *International Journal of Nanomedicine*, 2020, 15, 9025.  
<https://doi.org/10.2147/IJN.S276714>
- [15] H. N. K. Al Salman, E. T. Ali, M. Jabir, G. M. Sulaiman, & S. A. Al Jadaan. Benzhydrylsulfinyl N hydroxyacetamide Na extracted from fig as a novel cytotoxic and apoptosis inducer in SKOV 3 and AMJ 13 cell lines via P53 and caspase 8 pathway. *European food research and technology*, 2020.  
<https://doi.org/10.1007/s00217-020-03515-x>
- [16] A. G. Al-Ziaydi, A. M. Al-Shammari, M. I. Hamzah, H. S. Kadhim, & M. S. Jabir. Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. *VirusDisease*, 2020, 1-8.  
<https://doi.org/10.1007/s13337-020-00612-z>
- [17] S. Al-Musawi, S. Albukhaty, H. Al-Karagoly, G. M. Sulaiman, M. S. Jabir, & H. Naderi - Manesh. Dextran-coated superparamagnetic nanoparticles modified with folate for targeted drug delivery of camptothecin. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 2020, 11(4), 045009.  
<https://doi.org/10.1088/2043-6254/abc75b>
- [18] M. Jawad, K. Öztürk, & M. S. Jabir. TNF- $\alpha$  loaded on gold nanoparticles as promising drug delivery system against proliferation of breast cancer cells. *Materials Today, Proceedings*, 2021, 42, 3057-3061.  
<https://doi.org/10.1016/j.matpr.2020.12.836>
- [19] A. M. Al-Shammari, H. Al-Saadi, S. M. Al-Shammari, & M. S. Jabir. Galangin enhances gold nanoparticles as anti-tumor agents against ovarian cancer cells. In *AIP Conference Proceedings*, 2020, Vol. 2213, No. 1, p. 020206. AIP Publishing LLC.  
<https://doi.org/10.1063/5.0000162>
- [20] A. A. Ibrahim, M. M. Kareem, T. H. Al-Noor, T. Al-Muhimeed, A. A. AlObaid, S. Albukhaty, & U. I. Sahib. Pt (II)-Thiocarbohydrazone complex as cytotoxic agent and apoptosis inducer in Caov-3 and HT-29 cells through the P53 and Caspase-8 pathways. *Pharmaceuticals*, 2021, 14(6), 509.  
<https://doi.org/10.3390/ph14060509>
- [21] M. Jawad, K. Öztürk, & M. S. Jabir. TNF- $\alpha$  loaded on gold nanoparticles as promising drug delivery system against proliferation of breast cancer cells. *Materials Today, Proceedings*, 2021, 42, 3057-3061.  
<https://doi.org/10.1016/j.matpr.2020.12.836>
- [22] K. S. Khashan, F. A. Abdulameer, M. S. Jabir, A. A. Hadi, & G. M. Sulaiman. Anticancer activity and toxicity of carbon nanoparticles produced by pulsed laser ablation of graphite in water. *Advances in Natural Sciences*, 2020.  
<https://doi.org/10.1088/2043-6254/ab1de>
- [23] Z. S. Abbas, G. M. Sulaiman, M. S. Jabir, S. A. Mohammed, R. A. Khan, H. A. Mohammed, & A. Al-Subaiyel. Galangin/  $\beta$ - Cyclodextrin Inclusion Complex as a Drug-Delivery System for Improved Solubility and Biocompatibility in Breast Cancer Treatment. *Molecules*, 2022, 27(14), 4521.  
<https://doi.org/10.3390/molecules27144521>
- [24] A. M. Sameen, M. S. Jabir, & M. Q. Al-Ani. Therapeutic combination of gold nanoparticles and LPS as cytotoxic and apoptosis inducer in breast cancer cells. In *AIP Conference Proceedings*, 2020, Vol. 2213, No. 1, p. 020215. AIP Publishing LLC.  
<https://doi.org/10.1063/5.0000161>
- [25] W. H. Talib, A. I. Mahmood, D. Awajan, R. A. Hamed, & I. H. Al-Yasari. Immunomodulatory, Anticancer, and Antimicrobial Effects of Rice Bran Grown in Iraq: An In Vitro and In Vivo Study. *Pharmaceuticals*, 2022, 15(12), 1502.  
<https://doi.org/10.3390/ph15121502>
- [26] S. I. Ahmed, M. Q. Hayat, M. Tahir, Q. Mansoor, M. Ismail, K. Keck, & R. B. Bates. Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. *BMC complementary and alternative medicine*, 2016, 16(1), 1-9.  
<https://doi.org/10.1186/s12906-016-1443-z>
- [27] Z. He, B. Li, G. O. Rankin, Y. Rojanasakul, & Y. C. Chen. Selecting bioactive phenolic compounds as potential agents to inhibit proliferation and VEGF expression in human ovarian cancer cells. *Oncology Letters*, 9(3), 2015, 1444-1450.  
<https://doi.org/10.3892/ol.2014.2818>

- [28] F. Khan, P. Pandey, T. K. Upadhyay, A. Jafri, N. K. Jha, R. Mishra, & V. Singh. Anti-cancerous effect of rutin against HPV-C33A cervical cancer cells via G0/G1 cell cycle arrest and apoptotic induction. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders)*, 2020, 20(3), 409-418. <https://doi.org/10.2174/1871530319666190806122257>
- [29] S. N. Abd El Rahman. Rice Bran as a Functional Food. *Acta Scientific Women's Health (ISSN: 2582-3205)*, 2023, 5(3). <https://doi.org/10.31080/ASWH.2023.05.0473>
- [30] R. Ginwala, R. Bhavsar, D. G. I. Chigbu, P., Jain, & Z. K. Khan. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants*, 2019, 8(2), 35. <https://doi.org/10.3390/antiox8020035>

#### How to Cite

A. S. . Abdualhadi, "Study of the cytotoxic activity of alcoholic and aqueous extracts of Iraqi aromatic rice bran on B16 cell line", *Al-Mustansiriyah Journal of Science*, vol. 34, no. 4, pp. 37–44, Dec. 2023.

