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Cytotoxic Activity of Partial Purified Proteins *Saccharomyces Cerevisiae* and Lactic Acid Bacteria on HT-29 Cell Line

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Abstract

3- (4,5- dimethylthiazol - 2- yl) - 2,5- diphenyltetrazolium bromide (MTT) method is used to study the cytotoxicity effect of Partial Purified Proteins *Saccharomyces cerevisiae* and lactic Acid Bacteria on HT-29 Cell Line. The results showed all concentrations (6.25, 12.5, 25, 50,100 and 200µg/ml) of partial purified protein caused a significant inhibition rate of HT-29 after 72 hrs. Exposure and reached to 2.333, 9.667, 20.33, 37.00, 51.33 and 77.33 % respectively with statistically significant differences at P≤ 0.05 in comparing to control. While the result of cells viability (97.7, 90.3, 79.7, 63.0, 48.7and 22.7) % respectively when exposure (6.25, 12.5, 25, 50, 100 and 200 µg/ml) of Partial Purified Protein were used. Best fit value in HT-29 cell line is (37.39) µg/ml which represents a IC50 which is the lowest concentration that kills 50% of cells and the cells after treatment, noticed that they shrink, and their number and size reduced. From these results can concluded that the synergistic partial purified proteins of *S.cervesiae* and lactic acid bacteria have the effective role in treating cancer through its inhibitory effect And lethal toxicity on colon cancer cells line.

Keywords: Cytotoxic activity, Partial Purified Proteins, *Saccharomyces cerevisiae*, lactic Acid Bacteria, HT-29 Cell Line

Introduction

Cancer is a global health problem. According to the World Health Organization, there will be about 17 million deaths and 27 million new cases by 2030. It has been found that colon cancer is the most common, affecting more than one million people annually (Kumar *et al.* 2015) . 5% to 10% of infections are genetics, and the rest 90% -95% of cancers are due to infections, lifestyle and environmental toxins and pathogenic bacteria in the colon affect the process of neoplastic by inducing inflammation of the gastrointestinal mucosa (Floch *et al*. 2017 ; Eslami *et al*.2019). Scientists have found natural alternatives of treating cancer with low effectiveness and less side effects using by beneficial microorganisms as probiotics such as *Saccharomyces cerevisiae* and lactic acid bacteria(Porto *et al.* 2019). The beneficial microorganisms under anaerobic conditions produce short chain fatty acids (SCFA) such as acetate ,butyrate, propionate. These acids lower the pH and thus prevent the growth of pathogens which are sensitive to pH in nature. Also, these short-chain fatty acids act as a source of carbon for normal colonocyte. It may also reduce the proliferation of modified cells and stimulate the process of programmed cell death in the modified cells. Evidence has shown that four probiotic microorganisms (*L. salivarius FP25, L. salivarius FP35, P. pentosaceus, and E. faecium*) showed anti-proliferative properties. By direct attachment to colon cancer cells and the production of propionic and butyric short-chain fatty acids , production of free oxygen radicals and mitochondrial membrane permeability (Kumar *et al.* 2015).Lactobacilli prevent the growth and reproduction of cancerous cells ,through its cell wall and cytoplasmic extract to

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prevent cell cycle progression in S phase and showed an inhibitory effect on LT-97 and HT-29 cell (Eslami *et al.*2019). Also , previous studies have revealed the ability of *S. cerevisiae* to induce apoptosis in many cancer cell lines in the tongue, colon and breast without harming healthy cells (Seyfried *et al.* 2014). *S. cerevisiae* showed anti-cancer activity and included the apoptosis of HeLa cells decreased from 85% to 45% within 72 hours of treatment with 1000 µg / mL of yeast cells and showed signs such as cytomegalic, membrane hemorrhage and chromatin condensation(Rajan *et al.* 2017).In the study of Sambrani *et al.*, 2018 detected the cell free supernatant of *S. cerevisiae* showed showed stopped growing using HT-29 cell line after 48 h of treatment.

The current study was focused on the importance of utilizing backer's yeast dried powder product and lactic acid bacteria drug found in the markets and pharmacies of Baghdad – Iraq , and made from different origins ,which are considered beneficial microorganisms, as probiotic ,0n the other hand study the synergistic effects of baker's yeast *S. cervisiae* and lactic acid bacteria as anti-carcinogenic agent is considered the first record in this study.

Material and methods

Activation of *Saccharomyces cerevisiae*

S. cerevisiae (dry baker's yeast) from Saf instand yeast Turkish origin was obtained from Baghdad market and activated by suspending (0.1)g in (10)ml of warm sterile distilled water and incubated aerobically at 30°C for 30 min, mixed well by Vortex then streaked on Sabouraud Dextrose Agar (SDA) plates and incubated at 37°C for 48 hours , After the emergence of growth, part of the colonies were taken by the sterile loop , then streaked on Sabouraud Dextrose Agar plates (Karki *et al.*, 2019).

Activation and cultivation of Lactic Acid Bacteria

The dried commercial probiotic tablet of LAB product was aseptically weighed 1:10 diluted in sterilized medium De Man-Rose-Sharpe (MRS) broth, then shake well and incubated at 37°C for 24 h . After the emergence of growth, part of the colonies were taken by the sterile loop , then streaked on (MRS) agar plates (Succi *et al.*, 2014).

Partial Purification Proteins

Ammonium Sulphate Precipitation

Ammonium sulfate precipitation was used to obtain partial purified proteins of lactic acid bacteria and *S. cerevisiae* after growing on liquid medium Sabouraud Dextrose Broth (SDA) for yeast and Man Rogosa Sharpe (MRS) broth for lactic acid bacteria at 37°C for 14 days , (100ml) from Cell free supernatant LAB and *S. cerevisiae* was placed on magnetic stirrer device ,(40)g of ammonium sulfate was added gradually with continuous stirring under cooling condition for 15 minutes then kept in refrigerator at4^{o}C for 24 hrs . then added (20)g of ammonium sulfate and replicated the process again to obtain a saturation rate 100% , saturation of ammonium sulfate. The solution was centrifuged at 15000 rpm for 20 min . Discard the supernatant and collected the precipitate to dissolve in (2)ml of potassium phosphate buffer (PBS) and kept in refrigerator until use (Alcantara *et al.*, 2014; Lei *et al.*, 2019).

Dialysis

The Ammonium sulfate used for the concentration of the protein solution was removed by using

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dialysis bags with a cut-off separation rate (8).KDa to 12 kDa was carried out with PBS buffer at 4°C for 24 hours with stirring and changing PBS for four times to increase the efficiency of dialysis process then immersed the dialysis bags in sucrose powder to concentrate the partial purified proteins and kept in refrigerator until the use (Lei *et al.*, 2019).

Partial Purified Protein Concentration Measurement

Standard curve preparation according to Lowry's method was used as standard solution serial concentrations (10-100 µg / ml) of Stock solution of standard BSA (bovine serum albumin (Lowry et al.,1951; Kadhem ,2018).

Human colon carcinoma

HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology , passage No.13 , from a 44 years old Caucasian female . The HT-29 cell line (Sigma-USA) were obtained from (IBCBU) the Iraq biotech Cell Bank Unit.

Preparation and Maintenance of HT-29 Cultures

HT-29 was routinely cultured in 75ml falcon plates and incubated at 37˚C under standard conditions. The medium used for growing of the HT-29 cells was RPMI-1640 prepared as in the paragraph (3.2.2.2.5) . Cell suspension should be prepared to carry out tissue culture experiments according to (Freshney, 2010; Baqer , 2019):-

1. The cell line was monitored to obtain confluent monolayer.
2. The culture medium was withdrawn and disposed of, then washing of cells under aseptic conditions with PBS.
3. Three ml of Trypsin/EDTA (0.025%) solution was added, the flask should be turned over to envelop the monolayer completely, and was incubated at 37˚C for 3-5 min . The cells were disassembled and separated from the falcon plate.
4. RPMI-1640 medium (15ml) was added and the plate was incubated at 37˚C with 5% CO2.

Count Viable Cells

The counting of viable cells was performed according to (Darling and Morgan 1994) using Trypan blue stain exclusion technique. The dead cells take on the stain and are distinguished from living cells that are transparent . The method was as follows:

portion of the cell suspension (0.3 mL) was mixed with an equal volume of Trypan blue stain solution (4%).Then (10-20µl) of the cellular suspension was transferred to the edge of (hemocytometer chamber) , the slide was left for 1-2 minutes, and then the number of cells was counted in one square (1 mm^2) using light microscope under 40X.

Before performing any test on the cell line, its viability must be measured from the following equation. The cell concentration per ml and the total number of cells were also calculated (Freshney, 2010; Baqer , 2019 ;Khashan *et al,.* 2020 b ; Jabir *et al,.* 2020).

$C = n \times d \times 10^4$

C = Cell conce. (cell/ml), n = The number of counted cells, d = Dilution factor =10. And 10^4 = The inverse of the volume index

To calculate the percentage of viable cells , the following equation was used: -Cells viability (Before the test) % = (The number of unstained cells / Total number of cells) × 100

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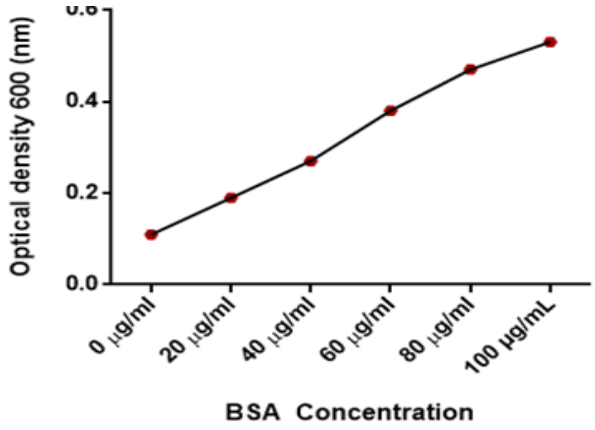
The HT-29 was recultured with Trypsin/EDTA twice a week, Monolayer confluence has reached 80%. Hence it is valid for toxicity testing (Iraqi centre for cancer researches and medical genetics / Baghdad –Iraq).

Partial Purified Protein concentration assessment

Partial Purified Protein of *S.cervisiae* and Lactic Acid Bacteria assessment was prepared according to (Lowry *et al.*,1951; Kadhem ,2018) ,four ml of solution No. (4) as described in (3.2.1.5.1) were added to (1 ml) of each BSA concentration and left at room temperature for 10 minutes. The blank contained only 1 mL of distilled water ,then 0.4 mL of Folin was added to each tube and left at 37^{o}C for 30 minutes. The optical density (OD) of the solutions was calculated at 600 nm . The standard curve is then drawn See (Figure 3-1)

Figure 3-1: Standard curve of BSA using Lowry method



To determine the protein concentration in the sample, 1 ml was taken and all the previous additions were added to it, the absorption was read at 600 nm, then the required protein concentration was determined according to the standard protein curve.

Cytotoxicity assay of Synergistic Partial Purified Proteins of *S.cervisiae* and Lactic Acid Bacteria on HT-29 Cell Lines

The cytotoxic effect of different concentrations of Partial Purified Proteins (*S. cervisiae* and LAB) on the proliferation of HT-29 cells in a 96-well plates, was carried out using MTT technique (3- 4, 5-dimethyl-2- thiazolyl-2, 5-diphenyl-tetrazolium bromide) and according to the method (Freshney, 2010;Al-Salman *et al*,.2020; Jabir *et al*,.2019).

1-The required concentrations was prepared for the partially purified protein(6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml,100 µg/ml and 200 µg/ml).

2- Cell suspension for cell line was prepared by shaking off the old culture medium and then adding 3 ml of Trypsin/EDTA(0.025%) solution to the flask (75ml) and incubated at 37ºC with 5% CO2 for 3-5 min.

3- Fifteen ml of a growth medium supplement was added with 10% fetal calf serum to form the cell suspension.

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4- Two hundred µl of cellular suspension (1 x 10^4 cells / ml) was placed in each well, the plate was sealed with a cap and incubated at 37 ° C with 5% CO_2 for 24 hours or until the cell reached a confluent monolayer.
5- The culture medium was removed and 200 µl of different protein concentrations were added to each well (3 replicates per concentration).
6- control wells (HT-29 cell line without protein treatment) (3-well replications) were treated with 200 µl of culture medium and the plate was sealed with a cap and incubated at 37° C with 5% CO_2 for 72 hours.
7- After the incubation period ended, the protein and culture medium was removed from the plate and washed with (PBS) to remove the separated (Non-living) cells.
8- Twenty-eight µl of MTT dye solution at (2 mg/ml) concentration was added to the whole well and incubated at 37°C with 5% CO_2 for 2.5hrs.
9- After the incubation period ends . The dye is carefully removed and 130 µL DMSO at 100% concentration are added to each well (to dissolve the remaining crystals in the well). The plate is left at 37°C for 15 minutes on a vibrating incubator (Al-Ziaydi *et al*,.2020).
10- The absorbency was determined on a micro-plate reader at 492 nm (The wavelength at which the highest absorption occurs) . The rate of inhibition or cytotoxicity of cell growth was calculated as follows (Khashan *et al*,.2019; Kareem *et al*,. 2020).
Inhibition (cytotoxicity) rate = A- B/A*100
A = the optical density of control
B = the optical density of the samples (Jabir *et al*,.2021 a)
*Note / The rate of viability cells after assay can be calculated from the following equation:
viability rate (after taking the test) % = 100 - Inhibition or cytotoxicity rate (Iraqi center for cancer researches and medical genetics / Baghdad –Iraq)

Microscopic examination of the Cancer cell line

To notice the morphological changes of the cancer cell line after treatment with Partial Purified Proteins (*S. cervisiae* and LAB) , the following steps were performed:
1-Two hundred µl of cellular suspension (1 x 10^5 cells / ml) was placed in (24-well plate) then it was sealed with a cap and incubated at 37°C with 5% CO_2 for 24h.
2- The culture medium was removed and 200 µl of protein concentrations (at lC50 concentration) were added to each well (The same amount of culture media is added to the control well) . The plate was sealed with a cap and incubated at 37° C with 5% CO_2 for 24 hours.
Note / the LC50, is the lowest concentration that kills 50% of cells (It is a value given by the micro-plate reader device)
3- After the incubation period ended, the protein and culture media was removed from the plate and washed by distilled water
4- Twenty µl of crystal violet stain was added to the each of wells and incubated at 37°C for 10-15 min (Khashan *et al*,.2019) .
5-The stain was carefully washed with tap water until the stain was completely removed. Cells were examined under an inverted microscope at 100X magnification and images were captured with a digital microscope camera (Waheeb *et al*,.2020; Al-Shammari *et al*,. 2020; Jabir *et al*,. 2021 b).

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Results and Discussion

Cytotoxic activity of Partial Purified Proteins on HT-29 cell line

The MTT assay (3- (4,5-dimethylthiazol -2-yl) -2,5-diphenyltetrazolium bromide) is a colorimetric assay for evaluating a cell's metabolic activity (Stockert *et al.*,2018). MTT assay was performed to determine the cytotoxicity of the partially purified protein. The test was done on the cancer cell line (HT-29) that represents the human colorectal adenocarcinoma cell line with epithelial morphology. In order to verify the inhibitory effect the partially purified protein was prepared with different concentrations. These concentrations were tested with three replicates on the cancer cell line. A comparison was made between before and after treatment and its relationship to the difference in the concentration of the substance under examination. A t-test was used to analyze the data. And find the value of IC50.

Table (1): Cytotoxic effect and cell viability of concentrations of Partial Purified Protein on HT-29 cell line

concentrations of Partial Purified Protein	Cytotoxicity (inhibition rate)%±SD	Cell viability %
control	0	100
6.25	2.333 ± 0.8819f	97.7a
12.5	9.667 ± 0.8819e	90.3b
25	20.33 ± 0.8819d	79.7c
50	37.00 ± 2.309c	63.0d
100	51.33 ± 1.856b	48.7e
200	77.33 ± 3.480a	22.7f

The result of HT-29 cell line (figure 1) explains the Inhibition Rate% during exposure to Partial Purified Protein . It revealed an effect on the cell line dependent on concentration, increasing of Protein amount leads to enhance the IR of the partial purified protein on the viability of HT-29 cells . The all treatments (6.25, 12.5, 25, ,50,100 and 200µg/ml) caused a significant inhibition rate reached to 2.333, 9.667, 20.33, 37.00, 51.33 and 77.33 % respectively with statistically significant differences at P≤ 0.05 in comparing to control . The data obtained were analyzed using an unpaired (t-test) with GraphPad Prism 6 (Khashan *et al*,.2020 b). Values were presented as mean ± SD of the triple measurements (Sameen *et al*,.2020).

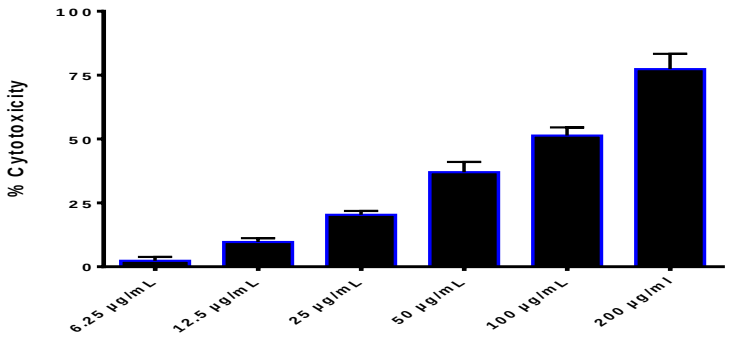

Figure (1) : Cytotoxic effect of Partial Purified Protein on HT-29 cells line .

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Figure (2) show the result of cells viability (97.7, 90.3, 79.7, 63.0, 48.7and 22.7) % respectively when (6.25, 12.5, 25, 50 ,100 and 200 µg/ml) of Partial Purified Protein were used . Best fit value in HT-29 cell line is (37.39) µg/ml which represents a IC50 which is the lowest concentration that kills 50% of cells.

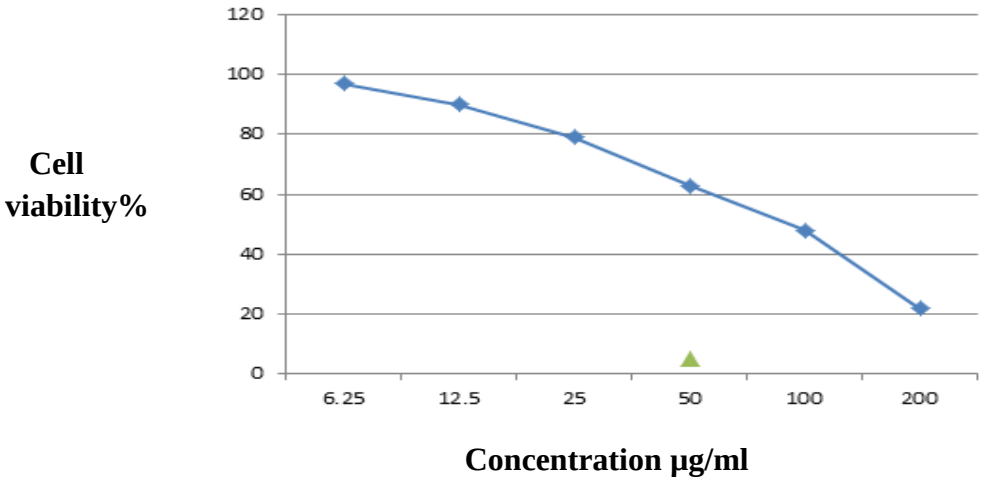


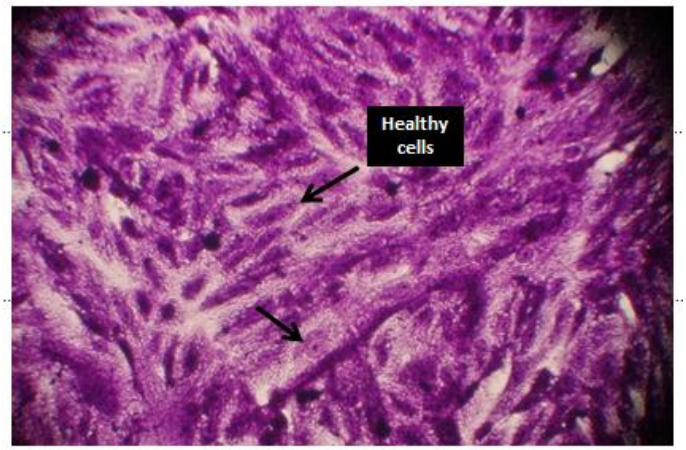
Figure (2) : Cell viability% effect of Partial Purified Protein in HT-29 cells. IC50=37.39 µg/ml

Microscopic examination

Examination before treatment showed swelling, agglutination, regularity in shape, and an abundance of cancer cells .See (figure 3).

When examining the cells after treatment, noticed that they shrink, and their number and size reduced. As shown in (figure 4).

Figure (3): Microscopic examination under an inverted microscope at 100X for the cancer cell line HT-29 , untreated



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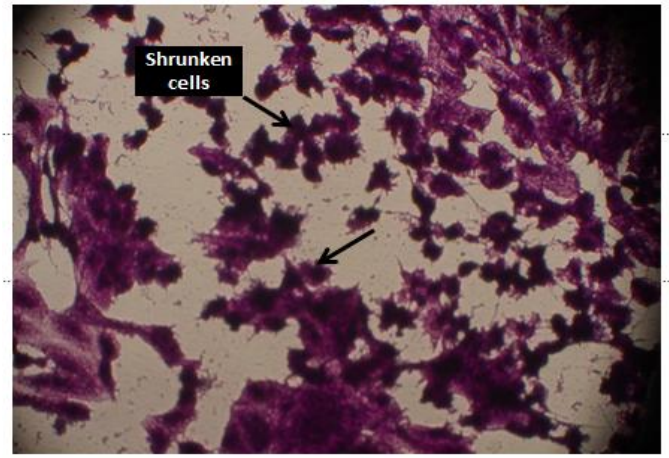

Figure (4) Microscopic examination under an inverted microscope at 100X for cancer cell line HT-29 , after 72hrs treatment of partial purified protein

The results indicate that the HT-29 cell line is very sensitive to partial purified protein of *S. cerevisiae* yeast and LAB at these concentrations . This study is consistent with previous studies that indicated a clear inhibitory effect of yeast and lactic acid bacteria on types of cancer lines .

Reported (Seyfried *et al.* 2014). That *S. cerevisiae* induces apoptosis on several cancer cell lines in the tongue, colon and breast without harming healthy cells.

Also, reported (Eslami *et al*.2019).That Lactobacillus cell wall and cytoplasmic extract prevent cell cycle progression in S phase and showed an inhibitory effect on LT-97 and HT-29 cell lines. And Bifidobacterium inhibits the growth of cancer cells by suppressing the cell cycle in the G0 / G1 phase . *Bacillus polyfermenticus* to, which has been used extensively since 1993 to treat intestinal disorders as it is able to produce bacteriocin and prevent the growth of several types of cancer cells such as breast, colon, lung and cervix.

Lactobacillus bacteria initiate apoptosis in cell strains HCT-116 and HT-29 by inducing Secretion of toxic metabolites and genetic changes on cancer cells (Sharma and Shukla, 2016).

References

1. Alcantara, V. A., Pajares, I. G., Simbahan, J. F., and Edding, S. N. (2014). Downstream Recovery and Purification of a Bioemulsifier from Saccharomyces cerevisiae 2031. The Philippine Agricultural Scientist, 96(4).
2. Al Salman, H. N. K.; Ali, E. T.; Jabir, M.;Sulaiman, G. M.& Al Jadaan, S. A. (2020). 2 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. Eurpean food research and technology.
3. Al-Ziaydi, A. G.; Al-Shammari, A. M.; Hamzah, M. I.; Kadhim, H. S., & Jabir, M. S. (2020). Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. VirusDisease, 1-8.
4. Al-Shammari, A. M.; Al-Saadi, H.; Al-Shammari, S. M. & Jabir, M. S. (2020). Galangin enhances gold nanoparticles as anti-tumor agents against ovarian cancer cells. In AIP Conference Proceedings (Vol. 2213, No. 1, p. 020206). AIP Publishing LLC.

5. Baqer , B. A.A. 2019 . Antibacterial, Immunomodulatory and Anticarcinogenic activities of Purified Arginine Deiminase from Lactobacillus pentosus , Ph.degree thesis – college of science – Mustansiriyah university .
6. Darling, D. C., & Morgan, S. J. (1994). Animal cells: culture and media. John Wiley & Sons.
7. Eslami,M.; Yousefi,B; Kokhaei,P; Hemati,M; Nejad,Z.R.; Arabkar,V.; Namdar,A.(2019): Importance of probiotics in the prevention and treatment of colorectal cancer. J. Cell Physiol. 1-17.
8. Floch, M. H.; Ringel,Y.; Walker, W. A. (2017): The Microbiota in Gastrointestinal Pathophysiology. Academic Press is an imprint of Elsevier,
9. Freshney, R. I. (2010). Database of misidentified cell lines. International journal of cancer, 126(1), 302-302.
10. Jabir ,M. S.; Saleh, Y. M.; Sulaiman, G. M. ;Yaseen, N. Y.; Sahib U. I. ; Dewir,Y. H.; Alwahibi,M.S. and Soliman, D. A. 2021a . Green Synthesis of Silver Nanoparticles Using Annona muricata Extract as an Inducer of Apoptosis in Cancer Cells and Inhibitor for NLRP3 Inflammasome via Enhanced Autophagy. Nanomaterials. 11, 384. 1-22.
11. Jabir, M. S.; Hussien, A. A.; Sulaiman, G. M.; Yaseen, N. Y.; Dewir, Y. H.; Alwahibi, M. S. & Rizwana, H. 2021b. Green synthesis of silver nanoparticles from Eriobotrya japonica extract: a promising approach against cancer cells proliferation, inflammation, allergic disorders and phagocytosis induction. Artificial Cells, Nanomedicine, and Biotechnology, 49(1), 48-60.
12. Jabir, M. S.; Nayef, U. M.; Abdulkadhim, W. K. & Sulaiman, G. M. (2019). Supermagnetic Fe3O4-PEG nanoparticles combined with NIR laser and alternating magnetic field as potent anti-cancer agent against human ovarian cancer cells. Materials Research Express, 6(11), 115412.
13. Jabir, M.; Sahib, U. I.; Taqi, Z., Taha; A., Sulaiman, G.; Albukhaty, S. & Rizwana, H. (2020). Linalool-Loaded Glutathione-Modified Gold Nanoparticles Conjugated with CALNN Peptide as Apoptosis Inducer and NF-κB Translocation Inhibitor in SKOV-3 Cell Line. International Journal of Nanomedicine, 15, 9025.
14. Karki, T. B., Timilsina, P. M., Yadav, A., Pandey, G. R., Joshi, Y., Bhujel, S., Adhikari, R., & Neupane, K. (2017). Selection and Characterization of Potential Baker's Yeast from Indigenous Resources of Nepal. Biotechnology Research International, 1–10.
15. Khashan , K. S.; Abdulameer, F. A.; Jabir, M. S.; Hadi, A. A, and Sulaiman, G. M. 2020a. . Anticancer activity and toxicity of carbon nanoparticles produced by pulsed laser ablation of graphite in water. Advances in Natural Sciences: Nanoscience and Nanotechnology, 11(3), 035010.
16. Khashan, K. S.; Jabir, M. S. & Abdulameer, F. A. (2019). Carbon Nanoparticles prepared by laser ablation in liquid environment. Surface Review and Letters, 26(10), 1950078.
17. Khashan , K. S.; Sulaiman, G. M.; Hussain, S. A.; Marzoog, T. R. and Jabir, M. S. 2020b . 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, 1-17
18. Kadhem , B.K.2018. Extraction, purification and characterization of bioemulsifier from Saccharomyces cerevisiae and its effect against breast cancer cell line , Ph.Degree thesis , department of biology –College of science / Mustansiriyah university .
19. Kumar,K.S.;Sastry,N. ; Polaki,H.; Mishra,V.(2015): Colon Cancer Prevention through Probiotics: An Overview. Cancer Science & Therapy 7(2) 081-092.
20- Lie, Sh. ; Zhao, R.; Sun,J. ; Ran, J. Ruan, X. and Zhu, Y. 2019. Partial purification and characterization of a broad-spectrum bacteriocin produced by a *Lactobacillus plantarum* zrx03

isolated from infant's feces , Food Science & Nutrition published by Wiley Periodicals, Inc., 2215-2222.

21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. Journal of biological chemistry, *193*, 265-275.
22. Porto,B.A.A.;Cinthia F. Monteiro,C.F.;Éricka L. S. Souza,E.L.;Leocádio,P.C.;Alvarez-Leite,J.; Gene roso,S;Cardoso,V.N.;Almeida-Leite,C.M.;Santos,D.A.Santos,J.R; Nicoli,J.R.;Pessione,E.;Martins,F. E.(2019),Treatment with selenium-enriched Saccharomyces cerevisiae UFMG A-905 partially ameliorates mucositis induced by 5-fuorouracil in mice Cancer Chemotherapy and Pharmacology 84:117–126.
23. Rajan,T.; Vincent,S.; Varghese,B.(2017) : saccharomyces cerevisiae-induced apoptosis of monolayer cervical cancer cells . Innovate Academic Sciences, Vol 10, Issue 8
24. Sambrani, R. ; Abdolalizadeh , J. ; Kohan , L. and Jafari , B. 2018. *Saccharomyces cerevisiae* inhibits growth and metastasis and stimulates apoptosis in HT-29 colorectal cancer cell line , springer journal , **28**: 85–995.
25. Sameen, A. M.; Jabir, M. S. & Al-Ani, M. Q. (2020). Therapeutic combination of gold nanoparticles and LPS as cytotoxic and apoptosis inducer in breast cancer cells. In AIP Conference Proceedings (Vol.2213, No.1, p. 020215).
26. Seyfried, T.N.; Flores, R.E.; Poff ,A.M.; Agostino, D.P.(2014) Cancer as a metabolic disease: Implications for novel therapeutics. Carcinogenesis, 35(3):515-2
27. Sharma, M.; Shukla, G. (2016). Metabiotics: One step ahead of probiotics; an insight into mechanisms involved in anticancerous effect in colorectal cancer. Frontiers in Microbiology, 7, 1940.
28. Stockert, J. C., Horobin, R. W., Colombo, L. L., & Blázquez-Castro, A. (2018). Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. Acta histochemica, 120(3), 159-167.
29. Succi, M ; Sorrentino, E. ; Renzo ,T. ; Tremonte, P.; Reale , A. ; Tipaldi ,L. ; Pannella , G. ; Russo,A. and Coppola, R. 2014. Lactic Acid Bacteria in Pharmaceutical Formulations: Presence and Viability of "Healthy Microorganisms , Journal of Pharmacy and Nutrition Sciences, 4, 66-75.
30. Waheeb, H. M.; Sulaiman, G. M., & Jabir, M. S. (2020). Effect of hesperidin conjugated with golden nanoparticles on phagocytic activity: *In vitro* study. In AIP Conference Proceedings (Vol. 2213, No. 1, p. 020217). AIP Publishing LLC.