Stallion Journal for Multidisciplinary Associated Research Studies
ISSN (Online): 2583-3340
Volume-3 Issue-4 || August 2024 || PP. 32-37 https://doi.org/10.55544/sjmars.3.4.4

Article Research: The Impact of Coliform Bacteria Co-Cultured Secondary Metabolites on Certain Cancer Cell Lines

Mohammed S. Abbas[1*], Esraa Jaafar Saheb[1], Athmar Jassim Mukhit[1] and Ali Abbas Kadhim[2]
[1]Department of Molcular biology, Iraqi Center for Cancer and Medical Genetic Research, Mustansiriyah University, Baghdad, IRAQ.
[2]High Institute for Infertility Diagnosis and ART's, Al-Nahrain University, Baghdad, IRAQ.

[*]Corresponding Author: mohammedsaleh@uomustansiriyah.edu.iq

www.sjmars.com || Vol. 3 No. 4 (2024): August Issue

Date of Submission: 25-07-2024	**Date of Acceptance:** 29-07-2024	**Date of Publication:** 29-08-2024

ABSTRACT

In order to avoid host defenses or maintain effective viral transmission as well as survival, viruses have developed a variety of strategies. Numerous DNA tumour viruses alter host DNA the enzymes methyl to cause epigenetic instability of hosting cells' immune-related genes regulation. Anti-tumor immune system responses were often associated with the host immunological mechanisms that were inhibited by virus-induced aberrant DNA methylation. In this instance, researchers discuss the processes including host-virus relationships that DNA tumor viruses utilize to control recipient Genome of their DNA with order to elude antimicrobial defense. This process might assist to create an immunological milieu that aids in the proliferation of malignancies. Positive outcomes are being seen in current immunotherapy investigations in treating various malignancies; yet, a considerable proportion of non-responders need the identification of new targeting for immunotherapy's against cancer. Consequently, knowing how cancer-causing pathogens evade the immunological system could potentially reverse immunological reduction to avoid or cure related malignancies.

***Keywords*-** esophageal cancer, *E. Coli*, *Klebsiella pneumoniae*, secondary metabolites.

I. INTRODUCTION

Cancer is the top cause of death across the globe. (Siegel et al., 2020). 9.6 million people were perished by various forms of cancer around the globe in 2018 (Dube et al., 2019). The rising number of fatalities due to cancer is anticipated to continue and reach 13.4 million deaths around 2030 (Lichtman 2013). Malignancy is characterized by unregulated cell proliferation, invasion, angiogenesis, immortalization, and metastasis (Chang et al., 2011). Throughout the previous decades, scientists continued to search for natural products from plants, animals, and microorganisms such as yeast, fungus, and bacteria and research their usefulness for cancer prevention and treatment (Tan et al. 2010).

A number of bacteria Produce a vast range of natural substances called secondary metabolites; some of these products have a good impact and offer great use in human and animal health. (Abdel Ghani et al., 2021). The gastrointestinal tract of humans is colonized by microorganisms, dominated by bacteria, of which 90% are Bacteroides and Firmicutes and the other 10% consist of many genera, including coliforms. The colony part of the intestine has the highest count of bacterial cells (10' bacteria/ml) (Coleman and Haller). Microorganisms living in unfavorable situations, like Coliforms, have been reported to exhibit the propensity to be producers and repositories of anti-cancer and anti-inflammatory substances (Safarpour et al., 2019). Actinomycetes are responsible for roughly 70% of secondary metabolites, Bacillus bacteria by 7%, and other bacteria by 1-2% (Khalifa et al. 2019).

Stallion Journal for Multidisciplinary Associated Research Studies
ISSN (Online): 2583-3340
Volume-3 Issue-4 || August 2024 || PP. 32-37 https://doi.org/10.55544/sjmars.3.4.4

In this work, we seek to identify the influence of secondary metabolites derived from coliform bacteria that have been cultivated under specified circumstances on the growth and development of cancer cells from various kinds of cell lines.

II. MATERIALS AND METHODS

Bacterial isolates and extraction of secondary metabolites

Isolates of coliform bacteria Escherichia coli and Klebsiella pneumonia were obtained from Al-Kindy teaching hospital and activated by culturing on nutrient agar for 24h at 37 C. Both E. coli. and K. pneumonia were inoculated together in nutrient broth as co-culture for the induction of competition and incubated for 72h at 37 C After 72h, the bacteria grown in nutrient broth were placed into ultracentrifuge for 15 min at 10000 rpm, bacterial cells settled down and the supernatant were taken and placed in sterilized glass tubes.

Preparation of dilutions from co-culture produced secondary metabolites

Four dilutions prepared by diluting of co-culture bacterial media (CCBM) with serum free media containing secondary metabolites as following; 1.75 ml RPMI SFM with 0.25 ml supernatant (25%) ,1 ml RPMI SFM with 1 ml of supernatant (50%), 1.5 ml RPMI SFM with 0.5 supernatant (75%), and 2 ml of the original CCBM as it is supernatant (0 dilution) table 1

Table 1. The serial of dilution prepared from co-culture produced secondary metabolites

V. co-culture bacterial media	V. RPMI serum free media	Dilution
0.25 ml	1.75 ml	2ml 25%
0.50 ml	1.50 ml	2ml 50%
0.75 ml	1.25 ml	2 ml 75%
2ml	0	2 ml As it is (0 dilution)

Cell line and cultures

Human esophagus Cancer cell line (SKG) supplied from the (experimental therapy department) in the Iraqi Center for Cancer and Medical Genetics Research -Mustansiriyah University (Baghdad, Iraq).

The cells were cultured in tissue culture vessels (Santa Cruz Biotechnology, California, USA) which contain media (RPMI-1640) with 10% fetal bovine serum (FBS), L-glutamine 1%, penicillin/streptomycin and were incubated at 37°C, 5% CO_2 for 24, 48, 72.

The attached monolayer cells in culture vessel were separated by adding 1 ml of trypsin/versine to form cell's suspended solution. Then added 10 ml from prepared media to this solution.

After that, 200 µl from the cells were cultured on sterile 96-well micro titer plate then let the cells for 24hr to make a monolayer to be treated with the extract of secondary metabolites

Treatment of the cells with bacterial extract dilutions.

After 24h, the monolayer cells in the 96-well micro titer plate were treated by adding 200 µl from the dilutions with 6 repeats for each dilution and 6 repeats of SKG cells without treatment as a control and incubated at 37C for 24h.

By using crystal violet assay, the plate was subjected for staining after 24h with crystal violet and were read by using ELISA reader.

III. STATISTICAL ANALYSIS

Data of current study were analyzed by using SPSS program with one-way ANOVA. The numeric data were described by Mean ± SD for cytotoxicity experiments. A level of significance of α=0.0001 was applied to test. Data of optical density were collected from the plate reader and non-linear regression analysis was used to determine the concentration of the extract necessary to create an IC50 of 50% reduction appeared in cell line.

IV. RESULT

The results illustrated in table (1) indicate 100% conc. bacterial crude had showed an effectivity on cancer cell line with viability rate 90.48 while 25%, 50%, 75% concentrations of the extracted crude showed no effectivity on cell line where the viability rate was 12, -49.34, -39.23 respectively.

The percentage of cell viability was calculated according to Betancur- Galvis and Gao (Rawa'a, 2018) as follow:
Viability rate = (mean of control-mean of treatment)/mean of control * 100%

Stallion Journal for Multidisciplinary Associated Research Studies
ISSN (Online): 2583-3340
Volume-3 Issue-4 || August 2024 || PP. 32-37 https://doi.org/10.55544/sjmars.3.4.4

Table 2. The viability of human esophagus cancer cell line under E. coli and Klebsiella pneumonia secondary metabolites extract exposure represented with mean and standard deviation

Seq.	Secondary metabolites Conc.%	Mean ± S.D	SKG Viability Rate
1.	Untreated (control)	0.841± 0.189	100
2.	25%	4.876 ±0.448	12
3.	50%	1.151±0.310	-49.34
4.	75%	1.338±0.123	-39.23
5.	As it is (0 dilution)	0.080**±0.008	90.48

SKG: human esophagus cell line
**(P< 0.0001), Significant

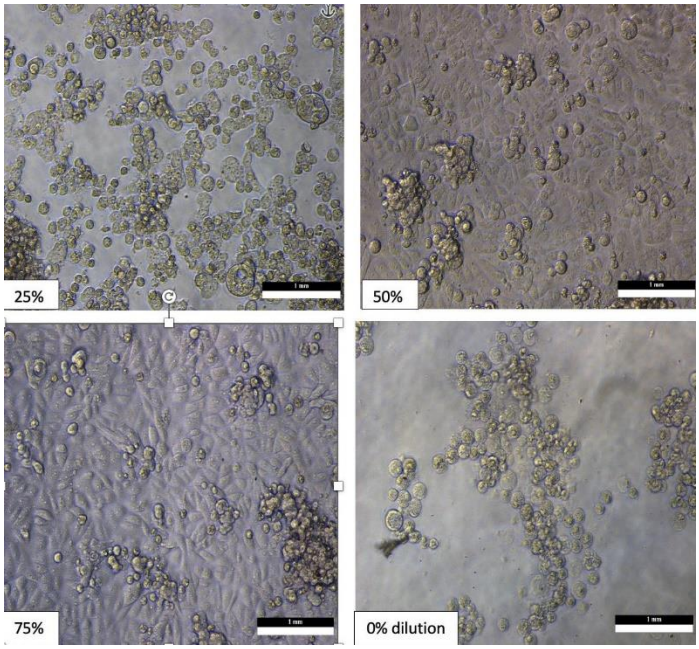

Figure 1. Represent SKG cell Line after 24hr treated with the bacterial CCBM before staining.

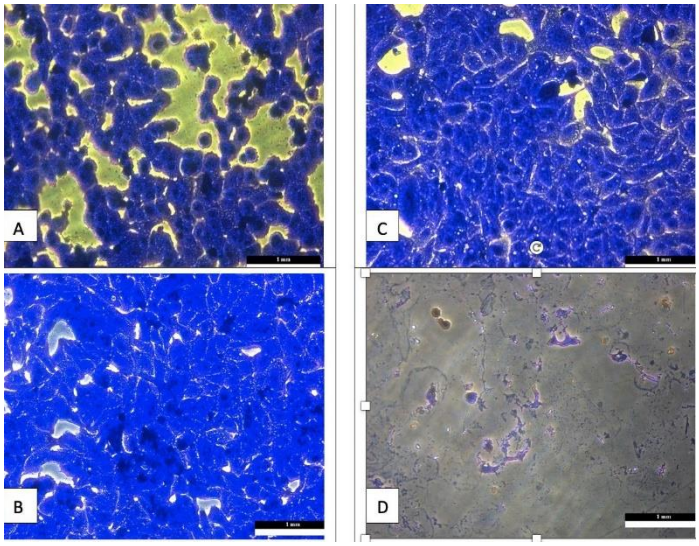

Figure 2-SKG cell line after treated with 0.25 ml (25%) of the crud
SKG cell line after treated with 0.50 ml (50%) of the crude
SKG cell line after treated with 0.75 ml (75%) of the crude
SKG cell line after treated with 1 ml (0 dilution) of CCBM

Stallion Journal for Multidisciplinary Associated Research Studies
ISSN (Online): 2583-3340
Volume-3 Issue-4 || August 2024 || PP. 32-37 https://doi.org/10.55544/sjmars.3.4.4

IC50 values of bacterial secondary metabolites extract on human esophageal cancer cell line

The study results showed that the bacterial crude extract has cytotoxic effect on esophagus cancer cells in a dose-dependent manner.

The inhibitory concentration of 50% of human esophagus cancer cell line (SKG) was 100% with IC50 value 4.7909, this result indicated that the extracted secondary metabolites bacterial crude has a high inhibition effect on human esophagus cancer cell and may showed a promising therapy for targeting cancer.

Cytotoxicity effect of CCBM on human esophagus cancer line

The lowest esophagus cancer cell viability appeared in the crude bacterial extract with concentration of 100% (0.080+_ 0.008) and it decreases with increasing the CCBM concentration to 25% (0.696+_ 0.189) as showed in table (1).

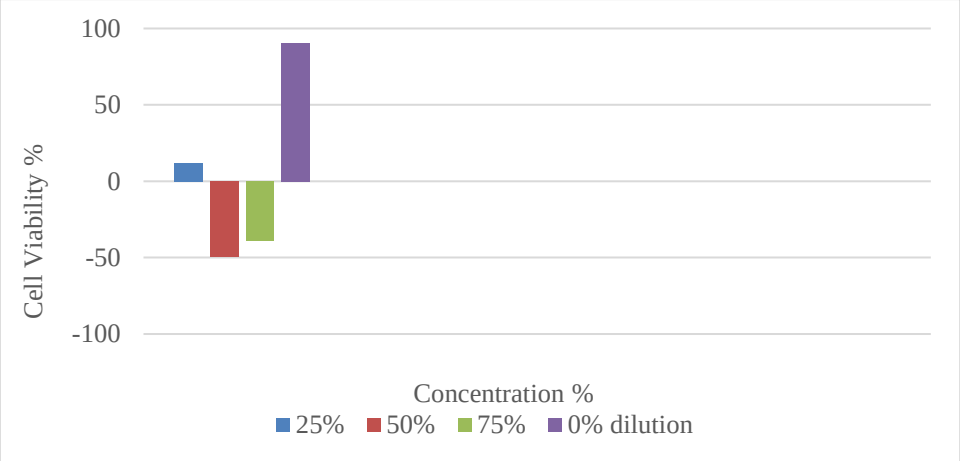

Figure 3. Represent the viability of human esophagus cancer cell line under bacterial extract exposure

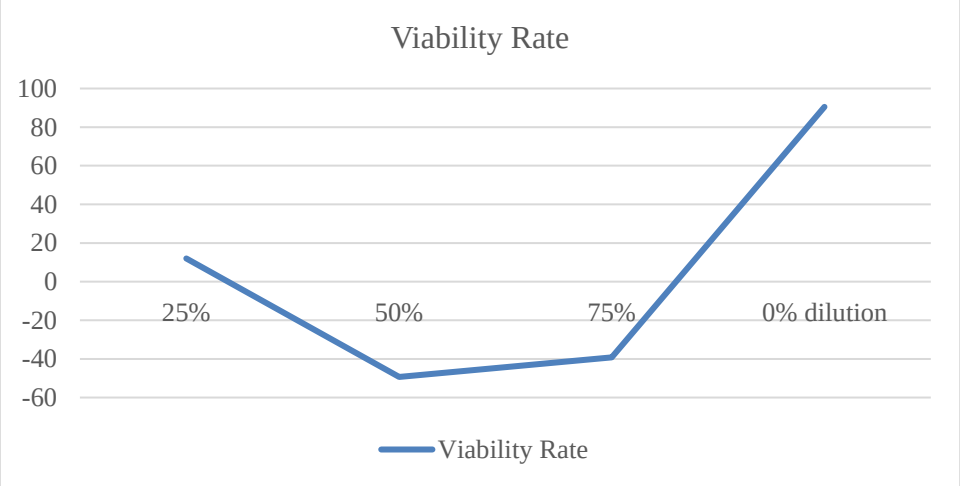

Figure 4. the viability of human esophagus cancer cell line under CCBM treatment

V. DISCUSSION

The bacterial kingdom has contributed enormously to the medical wellness of human beings and they are truly serving as a treasure house of bioactive secondary metabolites. Secondary metabolites and other components derived from bacteria have been used as antibiotics, anticancer drugs, antifungals, antiparasitic, and immunosuppressant agents for the past several decades. Consistent efforts have been put forth by researchers to identify new metabolites with good cytotoxic potential from various biological sources (Mohan et al., 2022). In this study, we experiment and discuss the possibility of using a bacterial secondary metabolites preparation isolated from E. coli and Klebsiella pneumoniae. in an attempt to reveal its effectiveness as an anti-cancer drug.

The main finding of this study that co-culture bacterial media (CCBM0) (0 dilution) had showed much higher activity on cancer cell with inhibition rate of about 90% compared with the other diluted preparations of the same CCBM.

However, the other preparations of the crude were diluted with RPMI serum-free media which may have contributed to losing the cytotoxic factor effectiveness by dilution. In the first diluted extract with 0.25 ml (25% of CCBM.) only about 12% of SKG cancer cells observed to have been inhibited, while the second and third diluted CCBM with 0.50 ml (50% of CCBM.), 0.75 ml (75% of CCBM.) showed no growth inhibition effect on cancer cells. In contrast, the fourth CCBM of 1 ml that represent the as it is concentration without any dilution (0 dilution) showed a significant high inhibition activity on SKG cancer cells growth of 90.48% ($P < 0.0001$) (0.080_+0.008) and that indicate an excellent cancer inhibitor that may represent the promising therapy for targeting cancer by mean of nature.

The results have supported many previous studies had been done on bacterial metabolites extracts that exhibit a powerful effect in eradication of cancer cells.

Previous study had been indicated that extracellular as well as intracellular extracts of the metabolites of thirty-six bacterial isolates shown cytotoxic potential on various cancer cell lines (Thomas et al.,2011). Several compounds with anti-cancer activity were identified by metabolites extraction of E. coli as well as anti-oxidant, anti-inflammatory, anti-diabetes, anti-microbial and anti-depressant compounds (Altaee et al., 2017). In addition, a numerous bioactive compound with powerful biological activity had been revealed in the metabolite's preparations extracted from Klebsiella pneumoniae, many with anti-inflammatory, anti-microbial, anti-cancer and cancer- preventive activity and much more with unknown pharmaceutical activity may represent the target of future studies (Al-Rubaye et al., 2017).

However, other studies reveals that some bacterial metabolites may induce tumor pathogenesis by promoting pro-inflammatory factors which stimulate airway epithelial cell proliferation, which ultimately induces cell transformation initiating tumor formation in some cancer e.g. lung cancer (Zhao et al., 2021). Moreover, some microbial components may confer a tumorigenic effect by directly affecting epithelial cells (oncogenes) (Ramirez-Labrada et al. 2020).

VI. CONCLUSION

The study concluded that bacterial secondary metabolites prepared from E. coli and Klebsiella pneumonia exert a great activity on human esophagus cancer cell line and showed a high cytotoxic effect in high dose of CCBM as it is compared to other diluted dilutions.

ACKNOWLEDGMENT

The research team extends its sincere thanks to the Iraqi Center for Cancer and Medical Genetics Research-Mustansiriyah University for provide support and facilities.

REFERENCES

[1] Abdelghani Z, Hourani N, Zaidan Z, Dbaibo G, Mrad M, Hage-Sleiman R. Therapeutic applications and biological activities of bacterial bioactive extracts. Arch Microbiol. 2021 Oct;203(8):4755-4776. doi: 10.1007/s00203-021-02505-1. Epub 2021 Aug 9. PMID: 34370077; PMCID: PMC8349711.

[2] Al-Rubaye, A. F., Kadhim, M. J., & Hameed, I. H. (2017). Characterization of antifungal secondary metabolites produced by Klebsiella pneumoniae and screening of its chemical compounds using GC-MS. International Journal of Current Pharmaceutical Review and Research, 8(2), 141-148.

[3] Altaee, N., Kadhim, M. J., & Hameed, I. H. (2017). Characterization of metabolites produced by E. coli and analysis of its chemical compounds using GC-MS. International Journal of Current Pharmaceutical Review and Research, 7(6), 13-19.

[4] Chang CC, Chen WC, Ho TF, Wu HS, Wei YH (2011) Development of natural anti-tumor drugs by microorganisms. J Biosci Bioeng 111(5):501–511

[5] Coleman OI, Haller D (2018) Bacterial signaling at the intestinal epithelial interface in infammation and cancer. Front Immunol 8:1927

[6] Khalifa SAM, Elias N, Farag MA, Chen L, Saeed A, Hegazy MF, Moustafa MS, Abd El-Wahed A, Al-Mousawi SM, Musharraf SG, Chang FR, Iwasaki A, Suenaga K, Alajlani M, Göransson U, El-Seedi HR (2019) Marine natural products: a source of novel anticancer drugs. Mar Drugs 17(9):491

[7] Lichtman SM (2013) Global initiatives to enhance cancer care in areas of limited resources: what ASCO members are doing and how you can become involved. Am Soc Clin Oncol Educ Book. https://doi.org/10.14694/EdBook_AM.2013.33.411

[8] Mohan, C. D., Rangappa, S., Nayak, S. C., Jadimurthy, R., Wang, L., Sethi, G., ... & Rangappa, K. S. (2022, November). Bacteria as a treasure house of secondary metabolites with anticancer potential. In *Seminars in cancer biology* (Vol. 86, pp. 998-1013). Academic Press.

[9] Ramirez-Labrada AG, Isla D, Artal A, Arias M, Rezusta A, Pardo J, Galvez EM (2020) The influence of lung microbiota on lung carcinogenesis immunity, and immunotherapy. Trends Cancer 6:86–97.

[10] Rawa'a, A. M. (2018). Cytotoxic activity of taraxacum officinale ethanolic plant extract against human breast cancer (MCF-7) cells and human hepatic (WRL-68) cells. Iraqi Journal of Cancer and Medical Genetics, 11(1).

[11] Safarpour A, Ebrahimi M, Shahzadeh Fazeli SA, Amoozegar MA (2019) Supernatant metabolites from halophilic archaea to reduce tumorigenesis in prostate cancer in-vitro and in-vivo. Iran J Pharm Res 18(1):241–253

[12] Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. CA Cancer J Clin 70(1):7–30. https://doi.org/10.3322/caac.21590

[13] Tan LT, Chan CK, Chan KG, Pusparajah P, Khan TM, Ser HL, Lee LH, Goh BH (2019) Streptomyces sp. MUM256: a source for apoptosis inducing and cell cycle-arresting bioactive compounds against colon cancer cells. Cancers 11(11):1742

[14] Thomas, A. T., Rao, J. V., Subrahmanyam, V. M., Chandrashekhar, H. R., Maliyakkal, N., Kisan, T. K., ... & Udupa, N. (2011). In vitro anticancer activity of microbial isolates from diverse habitats. *Brazilian Journal of Pharmaceutical Sciences*, *47*, 279-287.

[15] Zhao, Y., Liu, Y., Li, S., Peng, Z., Liu, X., Chen, J., & Zheng, X. (2021). Role of lung and gut microbiota on lung cancer pathogenesis. *Journal of Cancer Research and Clinical Oncology*, *147*(8), 2177-2186.