Archives of Ecotoxicology, Vol. 3, No. 2, pp. 32-38, 2021

Archives of Ecotoxicology

Journal homepage: https://office.scicell.org/index.php/AE

OPEN ACCESS

Anticancer Activity Assay of Nano-Fractional Compounds that Purified from Soil Actinomycetes

Mohammed Suhad*, Shiltagh Ruaa

Laboratory Investigation Department, Faculty of Science , University of Kufa , Iraq Alnajaf Governorat., Iraq

Article info
Received 20 February 2021
Revised 1 July 2021
Accepted 5 July 2021
Published online 18 August 2021

Regular article

Keywords:
Actinomycetes,
cytotoxicity,
nanoparticle liposome,
HPLC, GC-mass

Abstract

Background and objectives: Cancer remains a global problem of health, and has been recorded as one of the causes of death after heart disease Natural products from plants, the environment and microorganisms are leveraged for the purpose of fighting cancer. Actinobacteria have been recognized as main sources of bioactive natural products as early as in the 1950s, for which about half of the secondary metabolites revealed, including enzymes, antibiotics, immunosuppressive, and anti-tumor agents

Materials and methods: The methods of this study included isolated and identification of bacteria from soil samples and identified by morphology characters and biochemical test. Subjected extract of actinomycetes to HPLC purification then collected purified fractions then analyzed by GC-mass. After the fractions were mixed with liposome nanoparticles which tested activity on HT29 colon cancer cell line.

Results: The results of identification of bacterial isolates showed the colonies growing on a SNA medium were morphologically identified where the colonies were well-growth and had a gray color, not producing dyes in the medium. The results of the biochemical tests indicated that isolates were amylase, catalase, and gelatinase producing isolates and non-lipase producing, non H2S production and consuming urea, while the carbon consumption test indicated the isolates' ability to consume starch, glucose and sucrose respectively. While the results of preparative HPLC revealed that 4 fractions were collected with desired amounts of each compound when using fraction collector in depend on mobile phase system in analytical HPLC with (50 % HPLC-grade acetonitrile) at 254 nm and cycling up was employed to increase the separation efficiency. The chemical composition of the HPLC fractions using GC-MS showed the identification of many components example (Hexadeconic acid, Octadecanoic acid, ethyl ester and Fumaric acid). The results *of In vitro* anti-tumor cytotoxicity showed that all four nano purified fractions were applied on HT 29 colon cancer cells and exhibited significantly differences compared with control treatments of inhibition cells number, these data were used to calculate the values of IC50 (the inhibitory value of half the number for all nanofractions. the application of concentration with inhibition value and solved the equation to IC50 value were gained, which were (151.4,16.4 , 16.6 and 43.8 µg/ml) to four nano fractions respectively.

Conclusion: This study showed that the use of HPLC to purify the bacterial extract and then combine the purified fractions with the nanoparticles liposome has inhibited cancer cells with high efficacy.

1. Introduction

To date, cancer remains a global problem of health, and has been recorded as one of the causes of death after heart disease**(Are *et al.*,2019; Chalbatani *et al.*, 2019)**.There are many causes of cancer, including unhealthy lifestyles such as eating junk food, alcohol, smoking and losing physical fitness **(Tan *et al.*,2019; Limsui *et al.*, 2010).** Cancer treatment methods include surgery, radiotherapy, immunotherapy and chemotherapy **(Chalbatani *et al.*, 2019).** These techniques are individually useful in special cases and when linked, give more efficient treatment of the tumor.

Natural products from plants, the environment and microorganisms are leveraged for the purpose of fighting cancer**.**Roughly more than 60% of anti-cancer drugs are derived from these sources **(Cragg & Newman, 2009; Nobili *et al.*, 2019)**. In general, the term natural products refers to primary and secondary metabolic products, which are biologically active compounds with a low molecular weight less than 3000 Daltons produced by organisms that help them survival **(Kinghorn *et al.*, 2009– Zhang *et al.*, 2005).**

Natural products have the potential to inhibit cancer progression and reverse its progression **(Kaur *et al.*, 2011; Aravindaram& Yang, 2010).** Natural products are also an alternative solution to chemotherapy and its associated side effects such as heart failure, diarrhea, and others**.**Due to its high toxicity, it may lose specialization in treatment **(Tan *et al.*, 2015; Suter& Ewer, 2013).** Medical chemotherapy must be a specialist to get rid of a type of cancerous cell, but it cannot distinguish between normal and cancerous cells. However, most

*Corresponding author: suhadm.shiltagh@uokufa.edu.iq
https://doi.org/10.36547/ae.2021.3.2.32-38

of the presently used anticancer treatments tend to destroy cancer and normal cells **(Ser *et al*., 2015).** Cancer chemoprevention is similarly essential as an interference in carcinogenesis. These can be obstructive agents that stop neoplastic process or defeating agents that inhibit the progress of cancer cells' malignant phenotype **(Tan *et al*., 2013; Surh, 2003).** Thus, it is a continuing work to search for highly specific and potent chemotherapy agents from substitute sources for example microorganisms.

Actinobacteria have been recognized as main sources of bioactive natural products as early as in the 1950s, for which about half of the secondary metabolites revealed, including enzymes, antibiotics, immunosuppressive, and anti-tumour agents, are formed by actinomycetes **(Dharmaraj, 2010; Kemung *et al*.,2018).** The well known representative genus of class Actinobacteria is the Streptomyces, which accounts for over 70% of commercially beneficial antibiotics **(Lee *et al*., 2 015; Pimentel-Elardo *et al*., 2010)**. Furthermore, it is notable that 80% of actinobacterial natural products documented previously are derived from the genus Streptomyces **(Dharmaraj, 2010).**

Recently the nanoparticle liposome has become important as a drug delivery vehicles. These carriers consist of a lipid bilayer that is a hollow spherical shape occupied by an aqueous phase. Therefore, any compound can be encapsulated inside the liposome in one of the two parts if it is hydrophilic inside and if it is lipophilic within the bilayer liposome **(Singh *et al*., 2019).** This formulation can encapsulate more than one drug at the same time, protecting the encapsulated compound from hydrolysis and degradation. In addition, targeting the surface proteins on the cells and ligand on lipid bilayer shell have addition functional allowing targeted entry of liposome into the cell, by ligand receptors target. These ligands attach to cell receptors that are over-expressed in convinced diseased cells, permitting entry of the drug through the cell membrane **(Eloy *et al*., 2014).**

Despite all of the above, studies regarding the potential biological effectiveness of actinomycetes metabolites as anti-cancer drugs and the discovery of new drugs are limited**.** In this respect, this study was developed as an attempt to discover the anticancer property from actinomycetes against Human colon adenocarcinoma cell lines.

2. Material and methods

2.1 Collection of Samples and Isolation of Actinomycetes

A 25 gm of agricultural soil sample was collected from a depth of 20 cm. It was placed in a sterile bag and transferred to the laboratory. For the purpose of isolating the Actinomycetes bacteria, the serial dilution method was implemented for the soil sample (1 gm of soil was diluted in 9 ml of distilled water and then 1 ml of this dilution was transferred. To 9 ml of distilled water and so on until the sixth dilution **(Williams et al., 1983).** 100 µl of the fourth and fifth dilutions were plotted on medium (starch nitrate agar (SNA)) prepared by (of 20 g/l starch, 1 g/L KNO3, 0.5 g/l K2HPO4, 0.5 g/l MgSO4.7H2O, 0.5 g/l NaCl, 0.01 g/l FeSO4, 15 g/l agar) and containing antifungal and bacterial agents (nystatin and naldixic acid) at 50 and 20 mg / l, respectively. The plates were then incubated at 30C for 7 days. The growth colonies were diagnosed morphologically and with some biochemical tests **(Aghamirian and Ghiasian, 2009; Reddy *et al.*, 2011)**.

2.2 Preparation of actinomycetes extract

Diagnosed isolates from Actinomycetes cultured on medium SNA At a degree of 28 c for a period of 7-14 days until the spors are completely formed. In a 250 ml flask containing medium ISP2 medium containing (4 g/l glucose, 4 g/l yeast extract, and 10 g/l malt extract,) was inoculated with spore suspension and incubated at 30 using a shaker incubator (150 rpm) for 15 days, the cells were separated using a centrifuge 5000 rpm and 4c and the extraction of cell biomass was with acetone and then the acetone was evaporated under the vacuum. The remaining water was extracted by acetyl acetate **(Shaaban *et al*., 2013).**

2.3 Separation and purification by HPLC technique

Shimadzu LC-6AD gradient pump, SPD-M20A prominence diode array detector, and HPLC 2D Chemstation software (Hewlett-Packard, Les Ulis, France). The chromatographic separation was performed with a reversed-phase column (Bio wide pare C18 ,25cm×4.6mm,5Mm supelco analytical column and Shim-pack prep-ODS 250×20 mml.D preparative column.).CBM-20A controller, DGU-20Adegaseos. FCR-10A, shimadzu fraction collector.

2.4 Development of separation protocol by analytical DAD-HPLC

The column temperature adjusted at 30 Cº at a flow rate of 1.0 ml/min to achieve the optimum resolution of the separation of many compounds .The injection volume was maintained at 20 µl of watery extract, the mobile phase had been employed to achieve the best separation condition was 50 % HPLC-grade acetonitrile

2.5 Fractionation protocol by preparative HPLC

The column temperature adjusted at 30 Cº at a flow rate of 10 ml/min to achieve the optimum resolution of the separation of compounds. The injection volume was maintained at 500 and 1000µl of extract.

2.6 Identification of chemical composition of the HPLC fractions using GC-MS

The collected fractions was analyzed by a coupled Varian gas chromatography/mass spectrometry (Perkin Elmer Auto XL GC, Waltham, MA, USA) equipped with a flame ionization detector to identify their chemical composition. The GC conditions were EQUITY-5 column (60 m 0.32 mm x 0.25 mm); H2 carrier gas; column head pressure 10 psi, the oven temperature was maintained initially at 70 C for 2 min, and then programmed from 70 to 250 C at a rate of 3 C/min. The ionization voltage was 70 eV and mass range m/z 39e400 amu. The identification of individual compounds was based on their retention times relative to those of authentic samples and matching spectral peaks available with the published data **(Iwasa *et al*., 2015).**

2.7 Nanoparticles- fractional mixture preparation

Nanoparticles Liposome Solution: It was ready prepared solution (according to Sigma Aldrich, Germany) and supplied in glass vial (0.4 mg) provided with nuclease free water (1ml) as a stock solution. The stock solution was diluted with adding distilled water in proportion of 100 µl of liposome: 900 µl D.W (4µg/ml). Each purified fractions at a concentration of 500 µg/0.5ml mixed with 0.5ml of Liposome. The proportion of 100 µl of liposome (Stock Solution) 900 µl D.W.(4µg/ml).

2.8 *In vitro* Anti-cancer Cytotoxicity

Each nano purified fractions were evaluated for their cytotoxicity using tissue culture technique. HT29 (Human colon adenocarcinoma)cell line was kindly provided by the National Cell Bank of Iran (NCBI), Pasteur Institute of Iran. Cells were

maintained in RPM1medium with 10% fetal calf serum, sodium pyruvate, 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C and 5% CO2 till the cytotoxicity bioassay was carried out. The potential cytotoxicity of nano purified fractions was tested using the method of Alley **et al. (1988)**. Briefly, 100 cells/well were plated onto 96-well dishes overnight before the treatment with the tested compounds to allow the attachment of cells to the wall of the plate. Different concentrations of each tested compound (0, 15.6, 31.25, 62.5, 125, 250 and 500µg/ml) were added to the cell monolayer and triple wells were used for each individual dose. Monolayer cells were incubated with the tested agent(s) for 48 h at 37°C and 5% CO2. At the end of the incubation period, Crystal violet (C.V) assay was used to determine the optical density of the cell growth in each well of the microtiter plate, by using plate reader. After the end point of cytotoxicity assay, the maintenance medium with the test substance was discarded out and the wells washed with 100 µl of cold PBS by automatic pipette. Then the cell cultures were fixed with 10 % buffered formalin for 20 min at room temperature. Fixative solution was discarded and 100 µl of 0.1 % aqueous CV solution was added to each well. The samples were incubated at room temperature for 20 min with gentle shaking. After that the plates were washed by submersion in flowing tap water for 15 min. The plates were allowed to dry in the air and the absorbance was read at 570nm by a microplate reader **(Castro-Garza *et al.*, 2007).**The percentage of inhibition was calculated according to the following equation: **(Chiang *et al.*, 2003)**

Inhibition Rate (I.R) % = (optical density of control wells - optical density of test wells) / (optical density of control wells) X 100.

The relation between surviving fraction and compound concentration was plotted to get the survival curve of each tumor cell line and the IC50. The concentration of an agent that causes a 50% growth inhibition, for each tested agent using each cell line was obtained from the survival curve **(Skehan *et al.*, 1990).**

3. Results

3.1 Identification of Actinomycetes isolates

The colonies growing on a SNA medium were morphologically identified where the colonies were well-growth and had a gray color, not producing dyes in the medium. The results of the biochemical tests indicated that isolates were amylase, catalase, and gelatinase producing isolates and non-lipase producing, non H2S production and consuming urea, while the carbon consumption test indicated the isolates' ability to consume starch, glucose and sucrose (Table 1).

Table 1 Morphological growth and biochemical tests of Actinomycetes isolates

Characters	Results
Growth on SNA medium	Gray color colony, no dyes production
Amylase production	+
Catalase production	+
Gelatinase production	+
Lipase production	-
H2S production	-
Urea decomposition	+
Starch utilization	+
Glucose utilization	+
Sucrose utilization	+

3.2 Fractionation by preparative HPLC

The results of preparative HPLC revealed that 4 fractions were collected with desired amounts of each compound when using fraction collector in depend on mobile phase system in analytical HPLC with (50 % HPLC-grade acetonitrile) at 254 nm and cycling up was employed to increase the separation efficiency, each fraction were collected at specific retention time (min) (Table 2).

Table 2 Fraction number and retention time (min) used preparation HPLC with separation system 50 % HPLC-grade acetonitrile

Fraction no.	Retention time
1	6.5
2	7.2
3	7.8
4	9.5

3.3 Chemical composition of the HPLC fractions using GC-MS

The chemical composition of four fractions which analysis by GC/MS showed the identification of many components, representing the major components in the each fractions arranged based on the retention time and area were showed in (Table 3) and (Figure 1).

Table 3 The chemical composition of four fractions

Fraction no.	Chemical composition	Rt.	Area %
1	Hexadeconic acid	10.362	52.42
	13-Docosenamide	32.256	7.003
	n-pentadecanoic acid ,trimethylsilylsilyl ester	47.808	9.52
2	Octadecanoic acid,ethyl ester	28.054	26.75
	2-benzothiazolecarboxaldehyde	5.910	2.55
	1-butamine,N-nitro-N-propyl	45.036	14.13
3	Trisiloxane ,1,1,3,3,5,5-hexamethyl	5.993	1.69
	Heaxdeconic acid,ethyl ester	22.013	44.56
	Octadecanoic acid,ethyl ester	27.902	32.20
4	Trisiloxane ,1,1,3,3,5,5-hexamethyl	6.014	26.47
	Butanedioc acid,dimethoxy-diethyl ester	45.054	112.72
	Fumaric acid,isopropyl tetradecylester	46.217	13.39

3.4 *In vitro* Anti-tumor Cytotoxicity

The results in (figure 2) showed that all four nano purified fractions were applied on HT 29 colon cancer cells and exhibited significantly differences compared with control treatments of inhibition cells number. Where the highest rate of inhibition of cancer cells was about 81.5, 89, 93.8 and 90.1%) for the nanofractions 1, 2, 3 and 4, respectively, which subjected with concentration 500 mg/ml. When using the half dilution series, the inhibition percentage decreased gradually with dose dependent response decreasing, And these data were used to calculate the values of IC50 (the inhibitory value of half the number for all nanofractions. the application of concentration with inhibition value and solved the equation to IC50 value were gained, which were (151.4, 16.4, 16.6 and 43.8 µg/ml) to four nano fractions respectively (Figure 2 and 3).

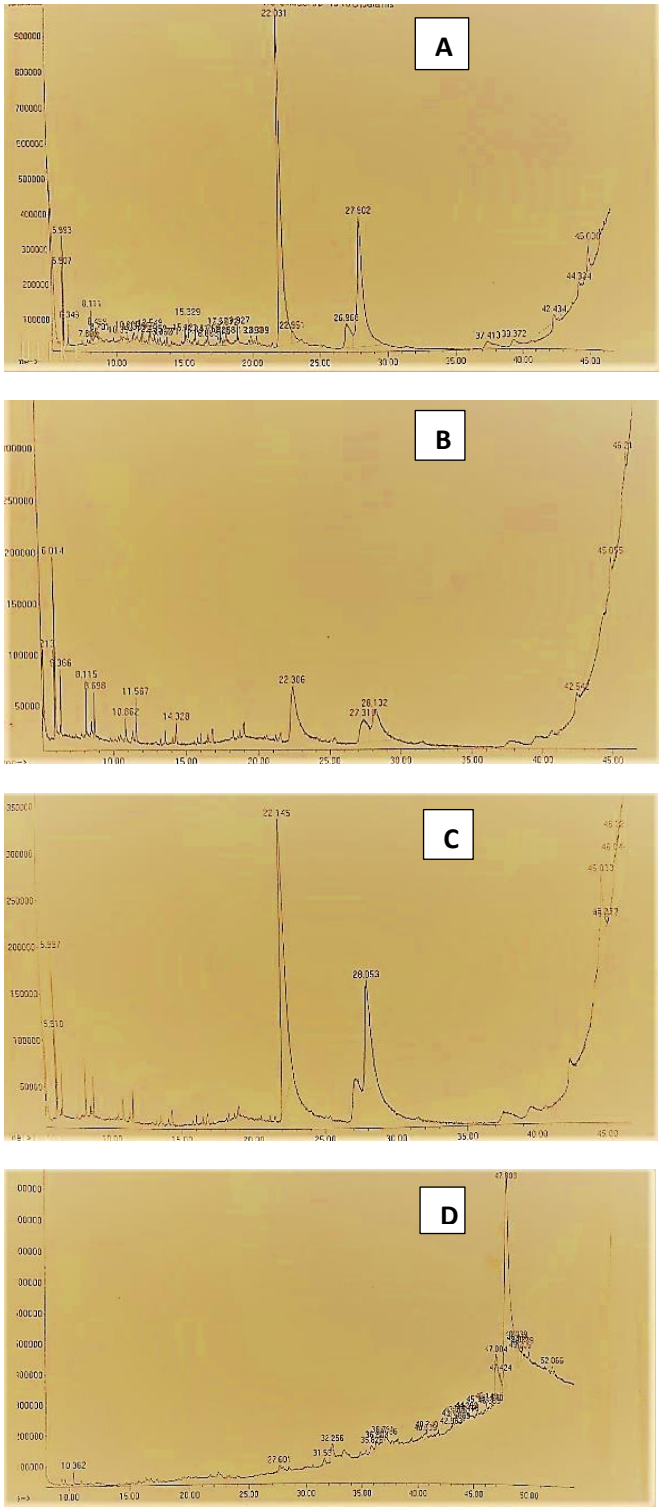

Figure 1 GC-MS chromatograph of Actinomycetes purified fractions (A-fraction 1, B-fraction 2, C-fraction 3and D-fraction 4)

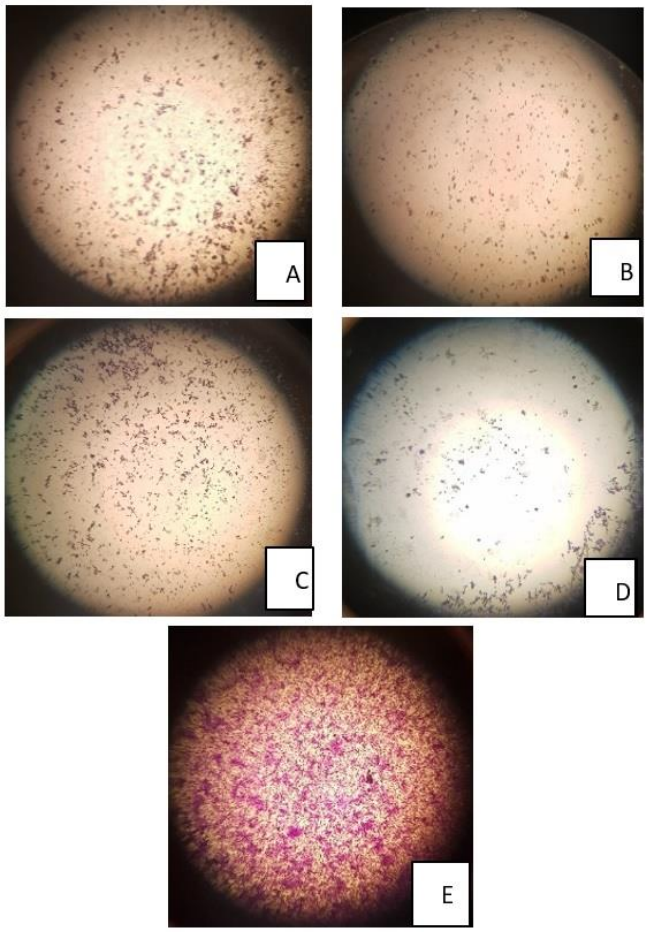

Figure 2 *In vitro* anti-cancer cytotoxicity assay of HT 29 colon cancer cell line subjected to A) fraction 1 nanoparticle 500 µg/ml. B- fraction 2nanoparticle 500 µg/ml c-fraction 3 nano particle500 µg/ml D- fraction 3nanoparticle 500 µg/ml and E-control HT 29 colon cancer cell line .

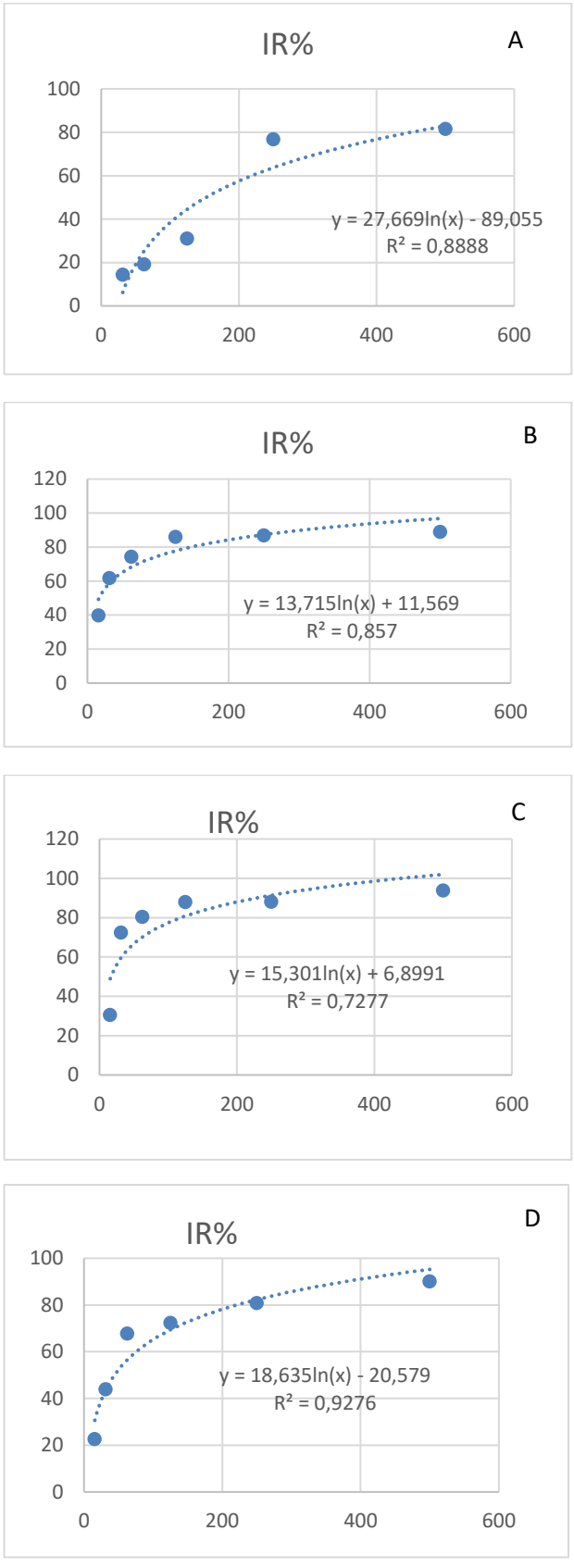

Figure 3 Dose response curve of growth inhibition of HT 29 colon cancer cell line when subjected to 1,2,3 and 4 nanoparticle fractions of actinomycetes (A,B,C and D)respectiveily presented by plotting of concentration versus IR% values.

4. Discussion

Actinomycetes is a Gram-positive, aerobic bacterium that is belonging to the order actinomycetales characterized by having an aerial mycelium. It is the most common filamentous organisms in the soil, and it is responsible for the smell of the earth, which indicates the vitality of the soil. It has a major role in the recycling of organic matter **(Bhatti and Bhat, 2017).**Actinomycetes is widespread in various habitats and participates in important processes, as it not only can live in harsh soil conditions such as lack of moisture and high salinity, but it stimulates plant growth. **(Hamdali *et al.*, 2008).**

In Georgia in the United States I refer to an example of filamentous bacteria prevalent in Pasture and cultivated soils (Lauber et al., 2009).In addition, **Burck *et al.* (2003)** indicated that Actinomycosis is the most common bacterial community in agricultural soils compared with forest soils when these soils were analyzed and compared in different countries. Moreover, he determined that actinomycetes increases after the transfer of lands from forest to agricultural **(Burck *et al.*, 2003; Fierer *et al.*, 2009)**.

Due to their biological importance and effectiveness, the secondary metabolic products of microbes have captured the interest of researchers, especially those that have an impact on human health. The biosynthesis of these products through engineering and biotechnology has shown significant benefits from conventional biomass extraction methods. Many types of soil bacteria produce unique secondary metabolic products that play important roles in many biological activities, the most important of which is Actinomycetes, which plays an important role in the manufacture of medicinal and pharmaceutical preparations due to the ability and effectiveness of these metabolic products and in various chemical compositions and Biological activities. Thousands of bioactive compounds have been isolated, diagnosed and developed from many different drugs to treat a wide range of human diseases, their poultry and their agriculture sectors **(Castillo *et al.*, 2002; El-Shatoury *et al.*, 2009).**

Actinomycetes is also a potential source of many metabolic by-products, antibiotics and other active compounds , It has a latent genetic potential to produce 10-20 secondary metabolites **(Bentley *et al.*,2002; Sosio *et al.*,2000).**There are evidence indicated it is a source of 75% of the compounds known as antibiotics, **(Nolan & Cross,1988; Thakur *et al.*,2009).** In addition to the antibacterial and antifungal the Streptomycetes produces anticancer drugs such as driamycin and the immunosuppressant tacrolimus **(Hopwood, 2007)** and contributes approximately 70% of the described metabolic products of filamentous bacteria **(Zengler *et al.*,2005).** Streptomycetes and other filamentous bacteria are useful sources of secondary metabolic products with numerous biological activities that may eventually be applied to the creation of effective anti-cancer agents and other beneficial pharmaceutical compounds **(Bibb, 2005).**

The chemical analysis of the purified fractions from the actinomycetes extract indicated the predominance of several compounds that may have the inhibition effect of cancer cells. Among them are compounds of the type of furan that **(Nguyen *et al.*, 2020)** indicated their anti-cancer ability when applied to cell lines of type (AGS, HCT116, A375M, U87MG, and A549) with IC50 values of 40.5, 123.7, 84.67, 50, and 58.64 µM, respectively.

It was also observed that Pentadecanoic acid has the effect of selective toxicity in MCF-7/SC comparison with parental cells. In addition, pentadecanoic acid inhibits the progressive and proliferative ability of cancer cells as indicated **(Nguyen *et al.*, 2020)** This is due to the ability of pentadecanoic acid to increase the gene expression of cancer cells to produce cleaved caspase-3, -7, -8, associated with the process of programmed cell death,

as increased production leads to apoptosis of cancer cell **(McIlwain *et al.*, 2015).**

A recent study demonstrates that heptadecanoic acid can exert anti-cancer effects on lung carcinoma cell line, emphasizing the efficacy of fatty acids in targeting human lung cancer cells **(Xu *et al.,* 2019).**

Also some researches showed that Fumaric acid used for inhibiting the solid growth of Ehrlich tumor in mice, was found to reduce markedly the growth and viability of Ehrlich, MH134, and L1210 mouse tumor cells in culture at concentration of 0.3 approximately 1.2 mg/ml**.(Kuroda and Akao, 1981) .**

There is no doubt that the use of nanocomposites as a catalyst in increasing target identification and ensuring intracellular access to drugs has been referred to in many studies one important example Nanoscale drug delivery systems using liposomes and nanoparticles are emerging technologies for the rational delivery of chemotherapeutic drugs in the treatment of cancer. Their use offers improved pharmacokinetic properties, controlled and sustained release of drugs and, more importantly, lower systemic toxicity **(Malam *et al.,* 2009).**

5. Conclusion

This study showed that the actinobacteria extract has very high efficacy against cancer cell lines of the type of colon cancer, as the compounds purified from the extract by HPLC, the chemical analysis of them by GC-mass showed they contain compounds that act to inhibit the cancer cells in addition to the increase in the effectiveness of these compounds. From its combination with liposomes nanoparticles that served to deliver the active substance into the cancer cell and destroy it.

Declaration of interest

The authors report no conflicts of interest.

References

1. Aghamirian, M. R., & Ghiasian, S. A. (2009). Isolation and characterization of medically important aerobic actinomycetes in soil of iran (2006-2007). *The open microbiology journal*, 3, 53.
2. Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., ... & Boyd, M. R. (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer research*, 48(3), 589-601.
3. Aravindaram, K., & Yang, N. S. (2010). Anti-inflammatory plant natural products for cancer therapy. *Planta medica*, 76(11), 1103-1117.
4. Are, C., McMasters, K. M., Giuliano, A., Yanala, U., Balch, C., Anderson, B. O., ... & Mori, M. (2019). Global forum of cancer surgeons: Perspectives on barriers to surgical care for cancer patients and potential solutions. *Annals of surgical oncology*, 26(6), 1577-1582.
5. Bentley, S. D., Chater, K. F., Cerdeño-Tárraga, A. M., Challis, G. L., Thomson, N. R., James, K. D., ... & Hopwood, D. A. (2002). Complete genome sequence of the model actinomycete Streptomyces coelicolor A3 (2). *Nature*, 417(6885), 141-147.
6. Bhatti, A. A., Haq, S., & Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant health. *Microbial pathogenesis*, 111, 458-467.
7. Bibb MJ. (2005) Regulation of secondary metabolism in Streptomyces. *Current Opinion in Microbiology*;8(2):208–215.
8. Burck, I.C., Yonker, C.M., Parton, W.J., Cole, C.V., Flach, K., Schimel, D.S. 1989. Texture, climate, and cultivation effects on soil organic matter content in USA grassland soils. *Journal of Soil Science Society of America*. 53, 800–805.
9. Castillo, U. F., Strobel, G. A., Ford, E. J., Hess, W. M., Porter, H., Jensen, J. B., ... & Yaver, D. (2002). Munumbicins, wide-spectrum antibiotics produced by Streptomyces NRRL 30562, endophytic on Kennedia nigriscansaaThe GenBank accession number for the sequence determined in this work is AY127079. *Microbiology*, 148(9), 2675-2685.]
10. Castro J., Barrios H. B., Cruz D. E., Said S., Carranza P., Molina C. A., and VeraL. (2007). Use of a colorimetric assay to measure differences in cytotoxicity of Mycobacterium tuberculosis strains. *Journal of medical microbiology*, 56(6), 733-737.
11. Cragg, G. M., & Newman, D. J. (2009). Nature: a vital source of leads for anticancer drug development. *Phytochemistry reviews*, 8(2), 313-331.
12. Dharmaraj, S. (2010). Marine Streptomyces as a novel source of bioactive substances. World *Journal of Microbiology and Biotechnology*, 26(12), 2123-2139.
13. Eloy, J. O., de Souza, M. C., Petrilli, R., Barcellos, J. P. A., Lee, R. J., & Marchetti, J. M. (2014). Liposomes as carriers of hydrophilic small molecule drugs: strategies to enhance encapsulation and delivery. *Colloids and surfaces B: Biointerfaces*, 123, 345-363.
14. El-Shatoury, S. A., El-Shenawy, N. S., & Abd El-Salam, I. M. (2009). Antimicrobial, antitumor and in vivo cytotoxicity of actinomycetes inhabiting marine shellfish. World *Journal of Microbiology and Biotechnology*, 25(9), 1547-1555.
15. Fierer, N., Carney, K.M., Horner-Devine, M.C., Megonigal, J.P. 2009. The biogeography of ammonia-oxidizing bacterial communities in soil. *Microbial Ecology*. 58, 435-445.
16. Hamdali, H., Hafidi, M., Virolle, M.J., Ouhdouch,Y. 2008. Growth promotion and protection against damping-off of wheat by two rock phosphate solubilizing actinomycetes in a P-deficient soil under greenhouse conditions. *Applied Soil Ecology*. 40, 510-517.
17. Hopwood, D. A. (2007). Therapeutic treasures from the deep. *Nature chemical biology*, 3(8), 457-458.]
18. Chalbatani, G. M., Dana, H., Memari, F., Gharagozlou, E., Ashjaei, S., Kheirandish, P., ... & Rouzbahani, F. N. (2019). Biological function and molecular mechanism of piRNA in cancer. *Practical laboratory medicine*, 13, e00113.
19. Iwasa, M., Nakaya, S., Maki, Y., Marumoto, S., Usami, A., & Miyazawa, M. (2015). Identification of aroma-active compounds in essential oil from Uncaria Hook by gas chromatography-mass spectrometry and gas chromatography-olfactometry. *Journal of oleo science*, 64(8), 825-833.
20. Kaur, R., Kapoor, K., & Kaur, H. (2011). Plants as a source of anticancer agents. *J Nat Prod Plant Resour*, 1(1), 119-124.
21. Kinghorn, A. D., Chin, Y. W., & Swanson, S. M. (2009). Discovery of natural product anticancer agents from biodiverse organisms. *Current opinion in drug discovery & development*, 12(2), 189.
22. Kuroda, K., & Akao, M. (1981). Antitumor and anti-intoxication activities of fumaric acid in cultured cells. Gann= Gan, 72(5), 777-782.
23. Lauber, C.L., Hamady, M., Knight, R., Fierer, N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*. 75, 5111-5120.
24. Lee, L. H., Zainal, N., Azman, A. S., Eng, S. K., Goh, B. H., Yin, W. F., ... & Chan, K. G. (2014). Diversity and antimicrobial activities of actinobacteria isolated from tropical mangrove sediments in Malaysia. *The scientific world journal*, 2014.
25. Limsui, D., Vierkant, R. A., Tillmans, L. S., Wang, A. H., Weisenberger, D. J., Laird, P. W., ... & Limburg, P. J. (2010). Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. JNCI: Journal of the National Cancer Institute, 102(14), 1012-1022.
26. Malam, Y., Loizidou, M., & Seifalian, A. M. (2009). Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. *Trends in pharmacological sciences*, 30(11), 592-599.
27. McIlwain, D. R., Berger, T., & Mak, T. W. (2015). Caspase functions in cell death and disease. Cold Spring Harbor Perspectives in Biology, 7(4).]
28. Nguyen, H. T., Pokhrel, A. R., Nguyen, C. T., Dhakal, D., Lim, H. N., Jung, H. J., ... & Sohng, J. K. (2020). Streptomyces sp. VN1, a producer of diverse metabolites including non-natural furan-type anticancer compound. *Scientific reports*, 10(1), 1-14.
29. Nobili, S., Lippi, D., Witort, E., Donnini, M., Bausi, L., Mini, E., & Capaccioli, S. (2009). Natural compounds for cancer treatment and prevention. *Pharmacological research*, 59(6), 365-378.25.
30. Nolan, R. D., & Cross, T. H. O. M. A. S. (1988). Isolation and screening of actinomycetes. *Actinomycetes in biotechnology*, 1-32.
31. Pimentel-Elardo, S. M., Kozytska, S., Bugni, T. S., Ireland, C. M., Moll, H., & Hentschel, U. (2010). Anti-parasitic compounds from Streptomyces sp. strains isolated from Mediterranean sponges. *Marine drugs*, 8(2), 373-380.]
32. Reddy, N. G., Ramakrishna, D. P. N., & Raja Gopal, S. V. (2011). A morphological, physiological and biochemical studies of marine Streptomyces rochei (MTCC 10109) showing antagonistic activity against selective human pathogenic microorganisms. *Asian J Biol Sci*, 4(1), 1-14.
33. Ser, H. L., Ab Mutalib, N. S., Yin, W. F., Chan, K. G., Goh, B. H., & Lee, L. H. (2015). Evaluation of antioxidative and cytotoxic activities of Streptomyces pluripotens MUSC 137 isolated from mangrove soil in Malaysia. *Frontiers in microbiology*, 6, 1398.

34. Shaaban, m., Abdel –Razic, A.S.,Abdel –Aziz,M.,Abozied,A.A.,Fadel,M.,2013. Bioactive secondary metabolites from marin Streptomyces albogriselus isolated from red sea coast. *J.Appl.Sci.Res*.9 (1), 996-1003.
35. Singh, A. K., Yadav, T. P., Pandey, B., Gupta, V., & Singh, S. P. (2019). Engineering nanomaterials for smart drug release: recent advances and challenges. Applications of targeted nano drugs and delivery systems, 411-449.
36. Sosio, M., Bossie, E., Bianchi, A., & Donadio, S. (2000). Multiple peptide synthetase gene clusters in actinomycetes. *Molecular Genetics and Genomics*, 264, 213-221.
37. Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. Nature *Reviews Cancer*, 3(10), 768-780.
38. Suter, T. M., & Ewer, M. S. (2013). Cancer drugs and the heart: importance and management. *European heart journal*, 34(15), 1102-1111.
39. Tan, G., Gyllenhaal, C., & Soejarto, D. D. (2006). Biodiversity as a source of anticancer drugs. *Current drug targets*, 7(3), 265-277.
40. Tan, L. T. H., Chan, K. G., Pusparajah, P., Yin, W. F., Khan, T. M., Lee, L. H., & Goh, B. H. (2019). Mangrove derived Streptomyces sp. MUM265 as a potential source of antioxidant and anticolon-cancer agents. *BMC microbiology*, 19(1), 1-16.
41. Tan, L. T. H., Ser, H. L., Yin, W. F., Chan, K. G., Lee, L. H., & Goh, B. H. (2015). Investigation of antioxidative and anticancer potentials of Streptomyces sp. MUM256 isolated from Malaysia mangrove soil. *Frontiers in microbiology*, 6, 1316.
42. Thakur, D., Bora, T. C., Bordoloi, G. N., & Mazumdar, S. (2009). Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by Streptomyces sp. 201. *Journal de Mycologie Medicale*, 19(3), 161-167.
43. To, N. B., Nguyen, Y. T. K., Moon, J. Y., Ediriweera, M. K., & Cho, S. K. (2020). Pentadecanoic Acid, an Odd-Chain Fatty Acid, Suppresses the Stemness of MCF-7/SC Human Breast Cancer Stem-Like Cells through JAK2/STAT3 Signaling. *Nutrients*, 12(6), 1663.
44. Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F., & van Sinderen, D. (2007). Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiology and molecular biology reviews*, 71(3), 495-548.
45. Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A., & Sackin, M. J. (1983). Numerical classification of Streptomyces and related genera. *Microbiology*, 129(6), 1743-1813.
46. Xu, C., Wu, P., Gao, J., Zhang, L., Ma, T., Ma, B., ... & Zhang, B. (2019). Heptadecanoic acid inhibits cell proliferation in PC-9 non-small-cell lung cancer cells with acquired gefitinib resistance. *Oncology reports*, 41(6), 3499-3507.
47. Zengler, K., Paradkar, A., & Keller, M. (2005). New methods to access microbial diversity for small molecule discovery. In *Natural Products* (pp. 275-293). Humana Press.
48. Zhang, L., An, R., Wang, J., Sun, N., Zhang, S., Hu, J., & Kuai, J. (2005). Exploring novel bioactive compounds from marine microbes. *Current opinion in microbiology*, 8(3), 276-281.