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 Al Najaf Governorat, Iraq

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 reveal, 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 % 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 compounds example (Hexadecanoic 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 HT29 colon cancer cell line.

**Conclusions:** 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.
of the presently used antitumor 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; Suhad et al., 2018). 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., 2015; Pimentel-Elardo et al., 2010). Furthermore, it is notable that 80% of actinobacterial natural products documented, are completely formed. In a 250 ml flask containing medium SNA At a degree of 28 c for a period of 7 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 pure C18 25cm×4.6mm,5Mm supco ko analytical column and Shim-pack prep-ODS 250×20 mmL 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 C 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 C 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-S 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 RPMI medium 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 (CV) 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 (LR) % = \frac{[\text{optical density of control wells} - \text{optical density of test wells}]}{[\text{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 concentration was plotted to get the survival curve of each compound (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            | 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            | Hexadecionic acid    | 10.362 | 52.42 |
|              | 13-Deocosenamide     | 32.256 | 7.003 |
|              | n-pentadecanoic      | 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          | 22.013 | 44.56 |
|              | hexamethyl           | 27.902 | 32.20 |
|              | Hexadecionic acid,ethyl ester | 32.20 | 6.014 |
|              | Octadecanoic acid,ethyl ester | 44.56 | 26.47 |
| 4            | trisiloxane          | 45.054 | 112.72|
|              | hexamethyl           | 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 nano fractions 1, 2, 3 and 4, respectively, which subjected with concentration 500 µg/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 3 and 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 2 nanoparticle 500 µg/ml, C) fraction 3 nanoparticle 500 µg/ml, D) fraction 3 nanoparticle 500 µg/ml and E) control HT 29 colon cancer cell line.
**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. (Handali 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 Streptomyces produces anticancer drugs such as dianmycin and the immunosuppressant tacrolimus (Hopwood, 2007). It also contributes approximately 70% of the described metabolic products of filamentous bacteria (Zengler et al., 2005). Streptomyces 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, HCT 116, 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.

**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) respectively presented by plotting of concentration versus IR% values.

\[
y = 27,669\ln(x) - 89,055 \quad R^2 = 0,8888
\]

\[
y = 13,715\ln(x) + 11,569 \quad R^2 = 0.857
\]

\[
y = 15,301\ln(x) + 6,8991 \quad R^2 = 0.7277
\]

\[
y = 27,669\ln(x) - 89,055 \quad R^2 = 0.857
\]
as increased production leads to apoptosis of cancer cell (Mcllwain 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 Aka, 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., Horsey, 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., & Yaing, N. S. (2010). Plantae medica

4. Bentley, S. D., Chater, K. F., Cerdóñ-Tárrega, A. M., Chaliss, 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.

5. Bhatti, A. A., Haq, S., & Bhat, R. A. (2017). Actinomycetes benefit role in soil and plant health. Microbial pathogenesis, 111, 458-467.

6. Bibb MJ. (2005) Regulation of secondary metabolism in Streptomyces. Current Opinion in Microbiology,8(2):208–215.

7. Burck, I.C., Yonker, C.M., Parton, W.J., Cole, C.V., Flach, K., Schimmel, 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.

8. 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 nigricansaThe GenBank accession number for the sequence determined in this work is AY127079. Microbiology, 148(9), 2675-2685.

9. 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.

10. Cragg, G. M., & Newman, D. J. (2009). Nature: a vital source of leads for anticancer drug development. Phytochemistry reviews, 8(2), 313-331.

11. Dharmaraj, S. (2010). Marine Streptomyces as a novel source of bioactive substances, World Journal of Microbiology and Biotechnology, 26(12), 2123-2139.

12. El-Shatoury, A. S., 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.

13. Fierer, N., Carney, K.M., Horner-Divine, M.C., Megonisig, J.P. (2009). The biogeography of ammonia-oxidizing bacterial communities in soil. Microbial Ecology, 58, 435-449.

14. Hamdali, H., Hafidi, M., Virolle, M.J., Oudhouch,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.

15. Hopwood, D. A. (2007). Therapeutic treasures from the deep. Nature - Microbiology, 3(8), 457-458.

16. Chalbatani, G. M., Duna, H., Memari, F., Ghargozoliou, E., Ashjai, S., Kheirandish, P., ... & Ronzubaham, F. N. (2019). Biological function and molecular mechanism of RNA in cancer. Practical laboratory medicine, 13, e00113.

17. Iwasa, M., Nakaya, S., Maki, Y., Matsunaga, 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.

18. Kaur, R., Kapoor, K., & Kaur, H. (2011). Plants as a source of anticancer agents. J Nat Prod Plant Resour, 1(1), 119-122.

19. 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.

20. Kuroda, K., & Aka, M. (1981). Antitumor and anti-intoxication activities of fumaric acid in cultured cells. Ganma Gan, 72(5), 777-782.

21. 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.

22. 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.

23. Limsu, 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.

24. 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.

25. Mcllwain, D. R., Berger, T., & Mak, T. W. (2015). Caspase functions in cell death and disease. Cold Spring Harbor Perspectives in Biology, 7(4).

26. Nguyen, H. T., Pokhrel, A. R., Nguyen, C. T., Dhakal, D., Lim, H. N., Jung, H. J., ... & Soling, J. K. (2020). Streptomyces sp. VNY1, a producer of diverse metabolites including non-natural furan-type anticancer compound. Scientific reports, 10(1), 1-14.

27. Nobili, S., Lippi, D., Wiort, E., Donmini, M., Bausi, L., Mini, E., & Capaccioli, S. (2009). Natural compounds for cancer treatment and prevention. Pharmacological research, 59(6), 365-378.25.

28. Nolan, R. D., & Cross, T. H. O. M. A. S. (1988). Isolation and screening of actinomycetes. Actinomycetes in biotechnology, 1-32.

29. 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.

30. Reddy, N. G., Ramakrishna, D. P. N., & Baja 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.

31. Ser, H. L., Ab Mutalib, N. S., Yin, W. F., Chan, K. G., Goh, B. H., & Lee, L. (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 marine Streptomyces albogriseus 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. 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.
42. 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.
43. 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.
44. 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.
45. 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.
46. 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.