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**RESEARCH ARTICLE**

# Fatty Acid Profile and In Vitro Anticancer Activity of Two Marine Sponge-Associated Bacteria

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**Short running title**: Anticancer potential of marine bacteria

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**ABSTRACT: Background:** Colorectal cancer represents one of prominent cause of mortality worldwide in men and women. The objective of this study was to search for new potential anticancer compounds, both in prevention and treatment of colorectal cancer. The anticancer potential of marine bacterial extracts against Human colorectal carcinoma cell line (HCT116) was evaluated as well as the partial identification of bioactive metabolites.

**Methods**: All bacterial extracts were tested for their cytotoxicity against HCT116 cell line by means of MTT assay. The highly cytotoxic dichloromethane extracts of marine sponge-associated bacteria *Vibrio* sp. and *Bacillus* sp. were analyzed by GC-MS

**Results**: Two fractions, Vib3 and Bac3, exhibited a very interesting cytotoxicity against human colorectal carcinoma (HCT116) cell line, with a percentage of cytotoxicity of 96.04 % and 29.48 %, respectively. The GC-MS analysis revealed the presence of two major fatty acids, palmitic and oleic acids, in Vib3 fraction and fatty acid esters and phenolic compounds in Bac3 fraction.

**Conclusion:** Based on previous literature, it may be hypothesized that the anticancer activity of bacteria extracts could be, at least partially, to the fatty acids fraction.

**Keywords:** Marine bacteria, Marine sponges, Fatty acids, Human colorectal carcinoma cell line (HCT116), GC-MS, cancer

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## 1. INTRODUCTION

Cancer still remains the most widespread cause of mortality in the world [1]. Therefore, the search of new drugs for the treatment of tumor disease represents a big challenge for many scientists. A lot of new molecules showing very interesting anti-proliferative and cytotoxic activities against cancer cells are provided by natural organisms (plants, bacteria, fungi) [2,3]. One of the most common cancer affecting men and women equally is colorectal cancer. The initiation and development of colorectal cancer are affected by genetic and/or by environmental factors [4,5]. The dietary patterns have been related to the initiation and treatment of colorectal cancer, in particular for those rich in fatty acids, especially PUFAs (polyunsaturated fatty acids) such as Mediterranean Diet.

The Mediterranean Diet is a unique healthy dietary model because its high nutraceutical foods (vegetables, fruits, olive oil) consumption rich of bioactive compounds such as polyphenols, carotenoids and fat [6,7,8]. Many experimental studies emphasize the importance of the consumption of olive oil as a protective agent in different diseases, in particular for colorectal cancer and other types of cancer [9,10]. This property has been ascribed to the presence of monounsatured fats, mainly oleic acid [11,12]. Although particular types of food represent the main source of fatty acids, microorganisms have been also considered of remarkable interest in the production of fatty acids, in particular PUFAs [13]. Marine bacteria, mainly the genus of *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, *Psychromonas*, *Vibrio*, and *Alteromonas*, are reported as great microbial producers of polyunsaturated fatty acids [14,15].

Herein we report the results of a study performed on marine sponge-associated bacteria belong to the genus of *Vibrio* and *Bacillus*. Our study provides a fatty acids profile on both microorganisms as well as the in vitro anticancer activity against human colorectal carcinoma cell line (HCT116) of dichloromethane fractions.

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## 2. MATERIALS AND METHOD

### 2.1. Isolation and identification of strains

Samples of *Dysidea avara* and *Ircinia variabilis* were collected in the bay of Naples at a depth of 20-25 m. The isolation and the identification of strains *Vibrio* sp. (from *D. avara*) and *Bacillus* sp. (from *I. variabilis*) were performed as previously reported [16,17]. Briefly, fresh marine sponges were transferred in laboratory and rinsed with sterile sea water. Then, the mesohyl tissues were recovered and inoculated in 20 mL of Marine Broth (Difco 2216). After 72h of incubation at 18 °C, bacterial cultures were plated on Marine Broth solid medium, and after 72h of incubation, colonies were picked up. Colonies were identified by means of 16s rRNA sequence analysis (DSMZ, Braunschweig, Germany) as strains of *Vibrio* sp. and *Bacillus* sp.

### 2.2. Extraction of secondary metabolites

Both strains were grown in Marine Broth at 30 °C for 72h. Next, the bacterial cultures were centrifuged and cell-free supernatants were recovered. The cell-free supernatant of *Vibrio* sp. and *Bacillus* sp. were extracted at first with diethyl ether to give Vib1 and Bac1 fractions, next with *n*-butanol to give Vib2 and Bac2 fractions and finally with dichloromethane to give Vib3 and Bac3 fractions.

### 2.3. Cytotoxic assay

Human colorectal carcinoma cell line (HCT116) was obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The cell line was cultured in McCoy's 5A medium (Sigma Aldrich Chemical Co., St. Louis. Mo. U.S.A) supplemented with 10% FBS (Fetal bovine serum), penicillin (100 $UmL^{-1}$) and streptomycin ($2mgmL^{-1}$) at 5% $CO_2$ in a 37 °C incubator. The cells were plated in 96-well plate at concentration of 5000 cell in 200 µL of medium per well. Tested fractions dissolved in DMSO were added to the wells in triplicates with final concentrations of 100 $\mu gmL^{-1}$ for 72h. 0.5% DMSO was used as negative control, while 2 µM staurosporine was used as positive control. The cytotoxic activity was determined using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as described by Mosmann, 1983 [18]. Briefly, medium was aspirated, 40 µl MTT salt ($2.5\mu gml^{-1}$) were added to each well and incubated for further 4 h. To stop the reaction and dissolve the formed crystals, 150 µl of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated over night at 37°C. The absorbance was then measured at 595 nm and a reference wavelength of 690 nm.

The equation used for calculation of percentage cytotoxicity was:

[1-(av(x))/(av(NC))]*100

Where: Av: average, X: absorbance of test fraction or compound, NC: absorbance of negative control.

### 2.4. Sample preparation for GC/MS analyses

Dried extract (2.5 mg) was prepared for chromatography by derivatization for 30 min at 85 °C with 15 µl pyridine + 20 µl N,O, bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and analyzed by GC/MS [19].

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### 2.5. GC/MS analyses

A Finnigan MAT SSQ 7000 mass spectrometer was coupled with a Varian 3400 gas chromatograph. DB-5 column, 30 m x 0.32 mm (internal diameter) was employed with helium as carrier gas (He pressure, 20 Mpa/cm$^2$), injector temperature, 310 ºC; GC temperature program, 85 – 310 ºC at 3 o C/ min (10 min. initial hold). The mass spectra were recorded in electron ionization (EI) mode at 70 eV. The scan repetition rate was 0.5 s over a mass range of 39 - 650 atomic mass units (amu).

The identification was accomplished by using computer search user-generated reference libraries, incorporating mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the bases of its mass spectral fragmentation. Reference compounds were co-chromatographed when possible to confirm GC retention times.

## 3. RESULTS AND DISCUSSION

Two strains belonging to *Vibrio* and *Bacillus* genus were isolated from marine sponges *Dysidea avara* and *Ircinia variabilis*, respectively. The extraction of cell-free medium of bacterial cultures with different solvents yielded six different fractions: Vib1 and Bac 1 (diethyl ether), Vib2 and Bac2 (*n*- butanol) and Vib3 and Bac3 (dichloromethane). All fractions were tested for their cytotoxic activity against human colorectal carcinoma cell line (HCT116) at a concentration of 100 ppm. Among all tested fractions, Vib3 extract showed impressive result (96.04 ± 0.825 %), while its counterpart (Bac3) gave weak result (29.48 ± 7.127 %) (Figure 1). In a previous work, the isolation and chemical characterization of compounds belonging to the diketopiperazine (DKP) class from cell-free supernatant of *Vibrio* sp. and *Bacillus* sp. have been described. Indeed, the dichloromethane fractions of the culture of *Vibrio* sp. and *Bacillus* sp. showed activation of Quorum Sensing both in TLC overlay and Lux screen assays. The isolated compounds that acted as signal molecules were identified as cyclo-(cis-4-hydroxy-D-prolyl-L-leucine) from *Bacillus* sp. and cyclo-(L-prolyl-L-phenylalanine) and cyclo-(L-prolyl-L-leucine) from *Vibrio* sp. [16]. The isolated DKPs did not show any cytotoxic activity against MCF7 cell line [20]. In the present study the highly active fractions, Vib3 and Bac3, against HCT116 cell line were further analyzed by means of GC-MS (Figure 2). All identified compounds belonged to chemical classes of fatty acids, fatty acid esters, terpenes and nitrogenous, phenyl and sulphur compounds (Table 1). Vib3 fraction was characterized by the presence of seven fatty acids, three of them with high percentage; palmetic acid (C16:0), oleic acid (C18:1) and *cis*-6-octadecenoicacid (C18:1) (28.9, 11.4 and 8.4% respectively). On the other hand, Bac3 fraction contained one fatty acid in very low concentration. More specifically, Bac3 exhibited high percentage of fatty acid esters (8,11-octadecadienoic acid, methyl ester (3.11%), 6-octadecenoic acid, methyl ester (4.76%), in addition to phenolic compounds (1,2-benzenedicarboxylic acid, bis(2-methylpropyl)ester (0.41%) and 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (0.42%) and terpenes which were present only in Bac3 fraction (Table 1).

A major compound (2-isobutoxyquinoline, 36.44%) was identified in Vib3 (Table 1, Figure 3).

The chemical composition of Bac3 fraction is reported in (Table 1, Figure 4).

It showed the presence of acetamide-2,2,2-trifluoro (36.17%) and 4-methyl-8-nitrooctane-3,5-dione (0.86%). The GC-MS analysis of both fractions revealed the presence of fatty acids as principal constituents, except for 2-isobutoxyquinoline and acetamide-2,2,2-trifluoro in Vib 3 and Bac 3, respectively. In particular, in Vib 3 fraction that exhibited the best cytotoxicity (96%) against HTC116 cell line, two major fatty acids, palmitic and oleic acids, were recognized. The cytotoxic activity of palmitic acid against adenocarcinoma cell line A549 in a concentration-dependent manner has been reported. Indeed, the treatment of A549 cells with palmitic acid resulted in a decrease in cell viability by inducing autophagy through topoisomerase I inhibition [21]. The interaction of palmitic acid with DNA topoisomerase-I was also observed in human colorectal carcinoma cells (HCT-116) showing a significant cytotoxic activity ($IC_{50}$ value of 0.8 μgmL$^{-1}$) [22]. A selective cytotoxicity of palmitic acid against human leukemic cells MOLT-4 at 50 μgmL$^{-1}$ by inducing apoptosis was also reported [23]. However, palmitic acid together with (Z)-9-octadecenoic acid and octadecenoic acid also showed anticancer potential on colon 26 tumor cells by targeting caspase-3 enzyme inducing apoptosis [24]. Moreover, oleic acid components present as major constituents of oil olive seemed to be responsible for the prevention of breast cancer [25].

Oleic acid (77.8%; 75.3%), linoleic acid (13.5%; 15.8%), palmitic acid (7.4%; 6.3%), were identified as the major constituents of almond oil from Northern Cyprus and Turkey, respectively. Both almond oils were active against Colo-320 and Colo-741 showing antiproliferative and anticancer activities. These activities were more similar in Colo-320 cells, which were treated with Northern Cyprus almond oil containing more fatty acids than that from Turkey [26]. The *in vitro* anticancer potential of fatty acids was also observed on human cervical cancer (HeLa) cells. The dichloromethane extract of *Clinacanthus nutans* showed a strong antiproliferative activity ($IC_{50}$ 70 μg/mL at 48 h) by inducing apoptosis with cell cycle arrest at the S phase. The composition of extract was determined by GC-MS analysis and the presence of several compounds, mostly fatty acids, was detected [27]. The GC-MS analysis of fraction Vib 3 revealed the presence of these fatty acids, in agree with reported data, the anticancer activity of this fraction could be attributed to the presence of these compounds.

Additionally, in Bac 3 fraction the 1, 2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester was detected. This known compound has been previously isolated from plants and microorganisms and it has been reported as anticancer compound on PC3, MCF-7, HCT116, A549, and MIAPACA cell lines, and this compound was proved to be a strong immunomodulatory B-cell stimulant [28,29]. Similar compounds (terpenoids derivatives) were identified in the methanolic extracts of *Eichhornia crassipes* (water hyacinth). The crude extract exhibited antioxidant activity and variable anticancer activities against four different cell lines: Human hepatocellular cancer cell line (HepG-2),

breast cancer cell line (MCF-7), cervix cancer cell line (HeLa) and Ehrlich Ascites Carcinoma Cells. Results showed that natural anticancer metabolites could affect cancer cells through their DNA damage by different mechanisms [30]. Therefore, the antiproliferative potential of Bac 3 on HCT116 could also be attributed to the presence of 1, 2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester and the identified terpenes.

## CONCLUSION

Fatty acids are key biocompounds involved in complex metabolic pathways, and carry out important biological roles (energy substrates, structural and functional components of cell membranes, precursors for lipid mediators, components affecting signal transduction pathways and gene transcription). In addition to their physiological functions, fatty acids have been reported as anticancer compounds in different in vitro models. The present paper reports further results on anticancer activity associated to bacterial extracts containing fatty acids. Then, this work supports the need for further studies on the use of fatty acids (pure or as crude extract) for drug development, cancer prevention and/or complementary agents.

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G.T and F.K.A.E.H conceived the experiments and wrote the manuscript; C.I., A.M.E.H and K.H.S. performed the chemical experiments (extraction and GC-MS); W.F. designed and carried out the cytotoxic assay. All authors read and approved the paper.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

**Availability of Data and Materials**: The data supporting the findings of the article is available in the [Supplementary materials] at [……..], reference number [S1, S2].

## REFERENCES

1. World Health Organization. Available online: https://www.who.int/news-room/fact-sheets/detail/cancer. Last accessed on 12th September 2018
2. Kalimuthu, S.; Venkatesan, J.; Kim, S.K. Marine derived bioactive compounds for breast and prostate cancer treatment: A review. *Curr. Bioact. Compd*., **2014**, *10(1)*, 62-74.
3. Saxena, S.; Chhibber, M.;Singh, I.P. Fungal bioactive compounds in pharmaceutical research and development. *Curr. Bioact. Compd.*, **2019**, *15(2)*, 211-231.
4. Theodoratou, E.; Timofeeva, M.; Li, X.; Meng, X.; Ioannidis, J.P.A. Nature, nurture, and cancer risks: Genetic and nutritional contributions to cancer. *Annu. Rev. Nutr*., **2017**, *37*, 293-320.
5. Yang, L.; Pei, Z. Bacteria, inflammation, and colon cancer. *World J. Gastroenterol*., **2006**, *12(42)*, 6741-6746.
6. Oliveira Raphaelli, C.; Gonçalves Azevedo,J.; Ollé Dalmazo,G.; Vinholes, J.; Braganhol, E.; Vizzotto, M.; Nora, L. Effect of fruit secondary metabolites on melanoma: A systematic review of *in vitro* studies. *Curr. Bioact. Compd.*, **2019**, *15(0)*, 1-27.
7. Dixon, L.B.; Subar, A.F.; Peters, U.; Weissfeld, J.L.; Bresalier, R.S.; Risch, A.; Schatzkin, A.; Hayes, R.B. Adherence to the USDA food guide, dash eating plan, and mediterranean dietary pattern reduces risk of colorectal adenoma. *J. Nutr*., **2007**, *137(11)*, 2443-2450.
8. Palozza, P., Mele, M.C.; Cittadini, A.; Mastrantoni, M. Potential interactions of carotenoids with other bioactive food components in the prevention of chronic diseases. *Curr. Bioact. Compd.*, **2011**, *7(4)*, 243-261.
9. Farinetti, A.; Zurlo, V.; Manenti, A.; Coppi, F.; Mattioli, A.V. Mediterranean diet and colorectal cancer: A systematic review. *Nutrition*, **2017**, *43-44*, 83-88.
10. Pérez-Martínez, P.; García-Ríos, A.; Delgado-Lista, J.; Pérez-Jiménez, F.; López-Miranda, J. Mediterranean diet rich in olive oil and obesity, metabolic syndrome and diabetes mellitus. *Curr. Pharm. Des*., **2011**, *17(8)*, 769-777.
11. Gill, C.I.R.; Boyd, A.; McDermott, E.; McCann, M.; Servili, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G.; McGlynn, H.; Rowland, I. Potential anti-cancer effects of virgin olive oil phenols on colorectal carcinogenesis models *in vitro*. *Int. J. Cancer*, **2005**, *117(1)*, 1-7.
12. Psaltopoulou, T.; Kosti, R.I.; Haidopoulos, D.; Dimopoulos, M.; Panagiotakos, D.B. Olive oil intake is inversely related to cancer prevalence: A systematic review and a meta-analysis of 13,800 patients and 23,340 controls in 19 observational studies. *Lipids Health Dis*., **2011**, *10*, 127.
13. Abd Elrazak, A.; Ward, A.C.; Glassey, J. Polyunsaturated fatty acid production by marine bacteria. *Bioprocess Biosyst. Eng*., **2013**, *36(11)*, 1641-1652.
14. Moi, I.M.; Leow, A.T.C.; Ali, M.S.M.; Rahman, R.N.Z.R.A.; Salleh, A.B.; Sabri, S. Polyunsaturated fatty acids in marine bacteria and strategies to enhance their production. *Appl. Microbiol. Biotechnol*., **2018**, *102(14)*, 5811-5826.
15. Gladyshev, M.I.; Sushchik, N.N.; Makhutova, O.N. Production of EPA and DHA in aquatic

ecosystems and their transfer to the land. *Prostaglandins Other Lipid Mediat*., **2013**, *107*, 117-126.
16. Abbamondi, G.R.; De Rosa, S.; Iodice, C.; Tommonaro, G. Cyclic dipeptides produced by marine sponge-associated bacteria as quorum sensing signals. *Nat. Prod. Commun*., **2014**, *9(2)*, 229-232.
17. De Rosa, S.; Mitova, M.; Tommonaro. G. Marine bacteria associated with sponge as source of cyclic peptides. *Biomol. Eng*., **2003**, *20(4)*, 311-316.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **1983**, *65(1)*, 55-63.
19. Christov, R.B.V.; Hegazi, A.; Abd El-Hady, F.; Popov, S. Chemical composition of egyptian propolis. *Z. Naturforsch. C*, **1998**, *53(3-4)*, 197-200.
20. Abd El-Hady, F.K.; Fayad, W.; Iodice, C.; El-Shahid, Z.A.; Abdel-Aziz, M.S.; Crudele, E.; Tommonaro, G. Investigating on the correlation between some biological activities of marine-sponge associated bacteria extracts and isolated diketopiperazines. *Curr. Microbiol*., **2017**, *74*, 6-13.
21. Karna, S.; Lim, W.B.; Kim, J.S.; Kim, S.W.; Zheng, H.; Bae, K.H.; Cho, M.S.; Oh, H.K.; Kim, O.S.; Choi, H.R.; Kim, O.J. $C_{16}$ saturated fatty acid induced autophagy in A549 cells through topoisomerase I inhibition. *Food Nutr. Sci.*, **2012**, *3 (9)*, 1220-1227.
22. Ravi, L. and Krishnan, K. Cytotoxic potential of N-hexadecanoic acid extracted from *Kigelia pinnata* leaves. *Asian J. Cell Biol*., **2017**, *12*, 20-27.
23. Harada, H.; Yamashita, U.; Kurihara, H.; Fukushi, E.; Kawabata, J.; Kamei, Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. *Anticancer Res*., **2002**, *22(5)*, 2587-90.
24. Yoo, Y.C.; Shin, B.H.; Hong, J.H.; Lee, J.; Chee, H.Y.; Song, K.S.; Lee, K.B. Isolation of fatty acids with anticancer activity from *Protaetia brevitarsis* larva. *Arch. Pharm. Res*., **2007**, *30(3)*, 361-365.
25. Tin Win, D. Oleic acid – the anti-breast cancer component in olive oil. *A.U.J. T*., **2005**, *9(2)*, 75-78.
26. Mericli, F.; Becer, E.; Kabadayı, H.; Hanoglu, A.; Yigit Hanoglu, D.; Ozkum Yavuz, D.; Ozek, T.; Vatansever, S. Fatty acid composition and anticancer activity in colon carcinoma cell lines of *Prunus dulcis* seed oil. *Pharm. Biol.*, **2017**, *55(1)*, 1239-1248.
27. Haron, N.H.; Md Toha, Z.; Abas, R.; Hamdan, M.R.; Azman, N.; Khairuddean, M.; Arsad, H. *In vitro* cytotoxic activity of *Clinacanthus nutans* leaf extracts against HeLa cells. *Asian Pac. J. Cancer Prev*., **2019**, *20(2)*, 601-609.
28. Save, S.A.; Lokhande, R.S.; Chowdhary, A.S. Determination of 1, 2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester from the twigs of *Thevetia peruviana* as a Colwell Biomarker. *J.I.A.P.S*., **2015**, *2(3)*, 349-362.
29. Krishnan, K.; Mani, A.; Jasmine, S. Cytotoxic activity of bioactive compound 1, 2-benzene dicarboxylic acid, mono 2-ethylhexyl ester extracted from a marine derived *Streptomyces* sp. VITSJK8. *Int. J. Mol. Cell. Med.*, **2014,** *3*, 246-254.
30. Aboul-Enein, A.M.; Shanab, S.M.M.; Shalaby, E.A.; Zahran, M.M.; Lightfoot, D.A.; El-Shemy, H.A. Cytotoxic and antioxidant properties of active principals isolated from water hyacinth against four cancer cells lines. *BMC Complement. Altern. Med*., **2014**, *14*, 397-397.

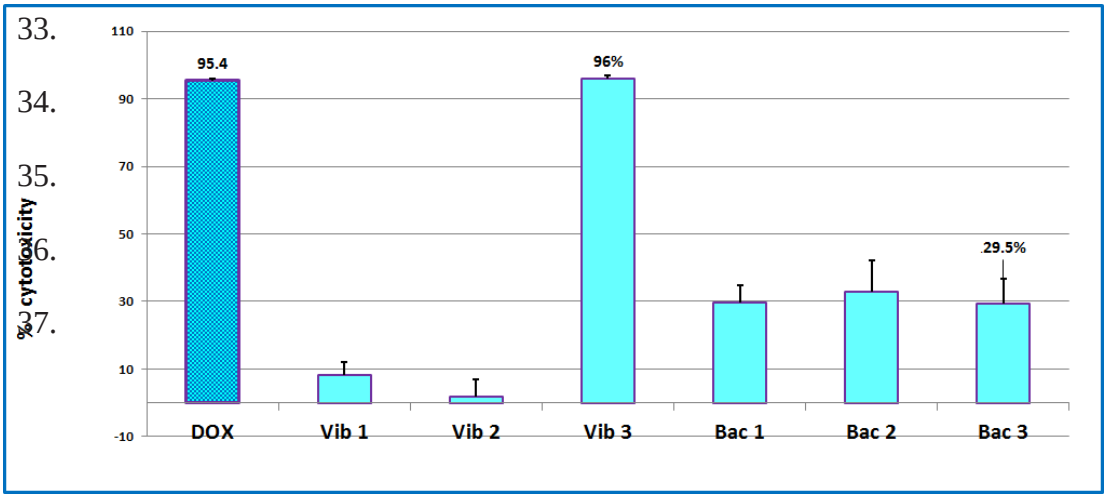


**Figure 1**. Average % cytotoxicity of Vibrio sp. (Vib 1-3) and Bacillus sp. (Bac 1-3) fractions against HTC116 colon carcinoma cell line. Values are expressed as mean ± SD, n = 3 at a concentration of 100 ppm

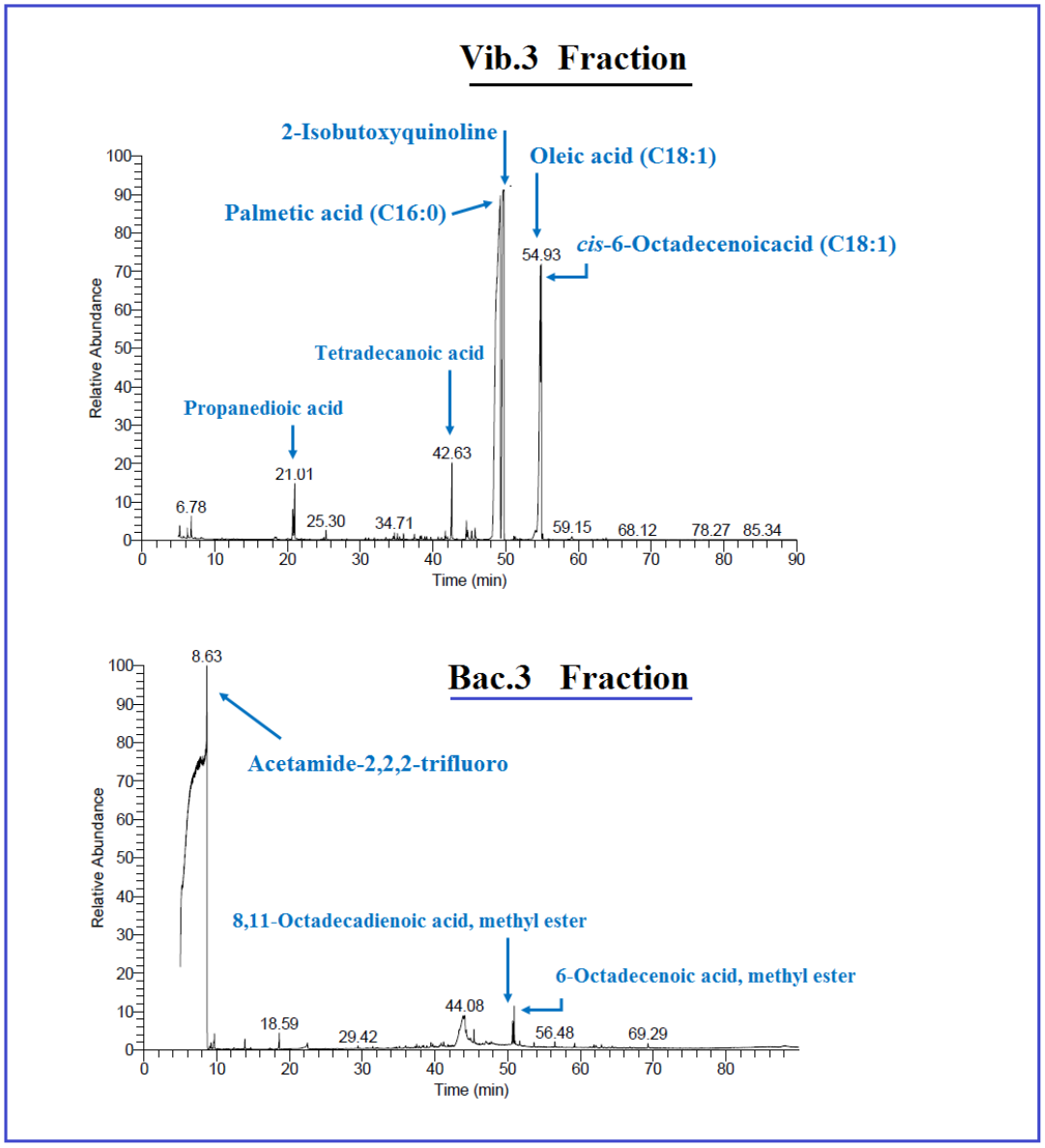


Figure 2. GC/MS chromatograms for Vib.3 and Bac.3 fractions

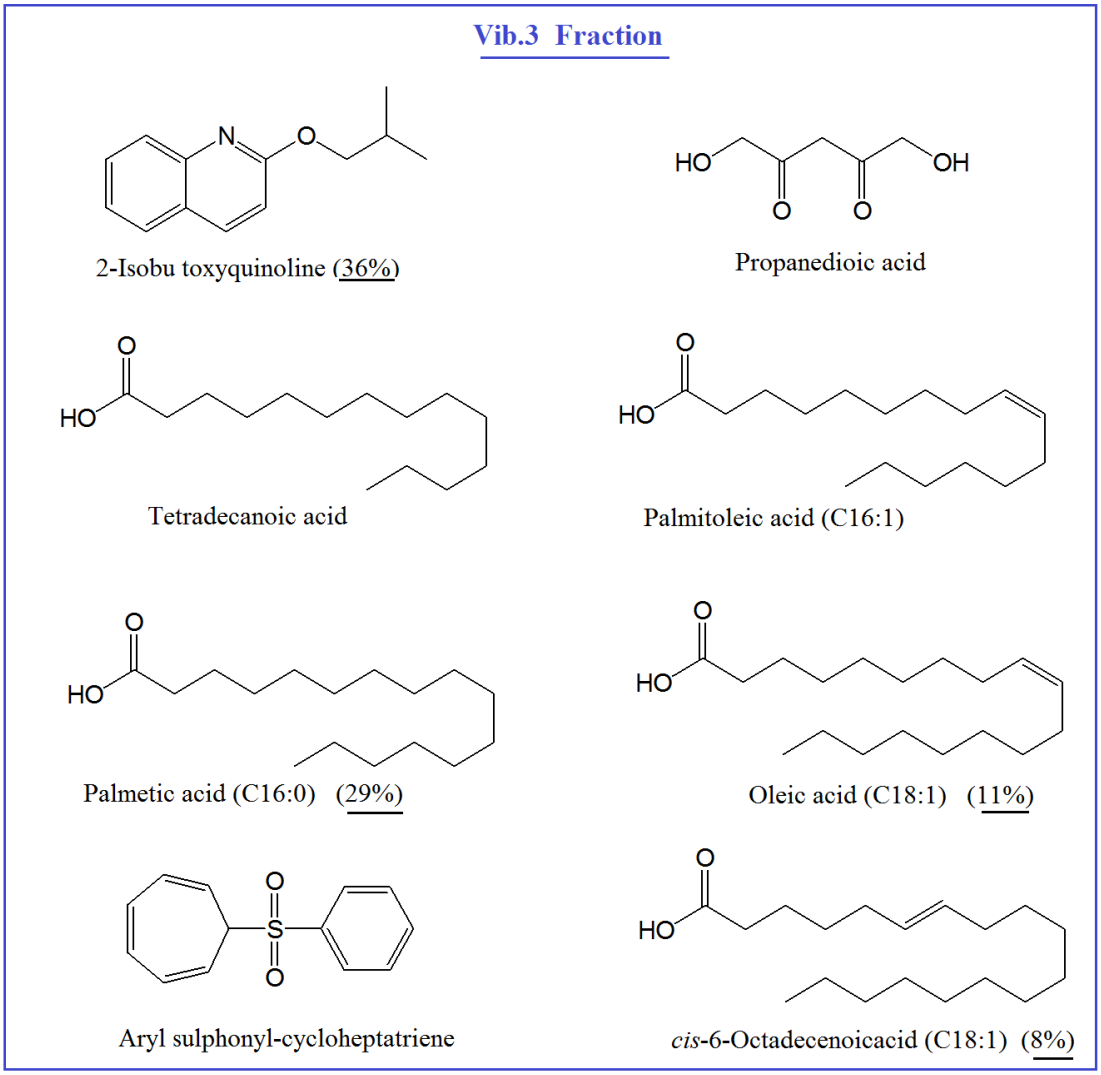


Figure 3. Major compounds identified by GC/MS analysis in Vib.3 fraction

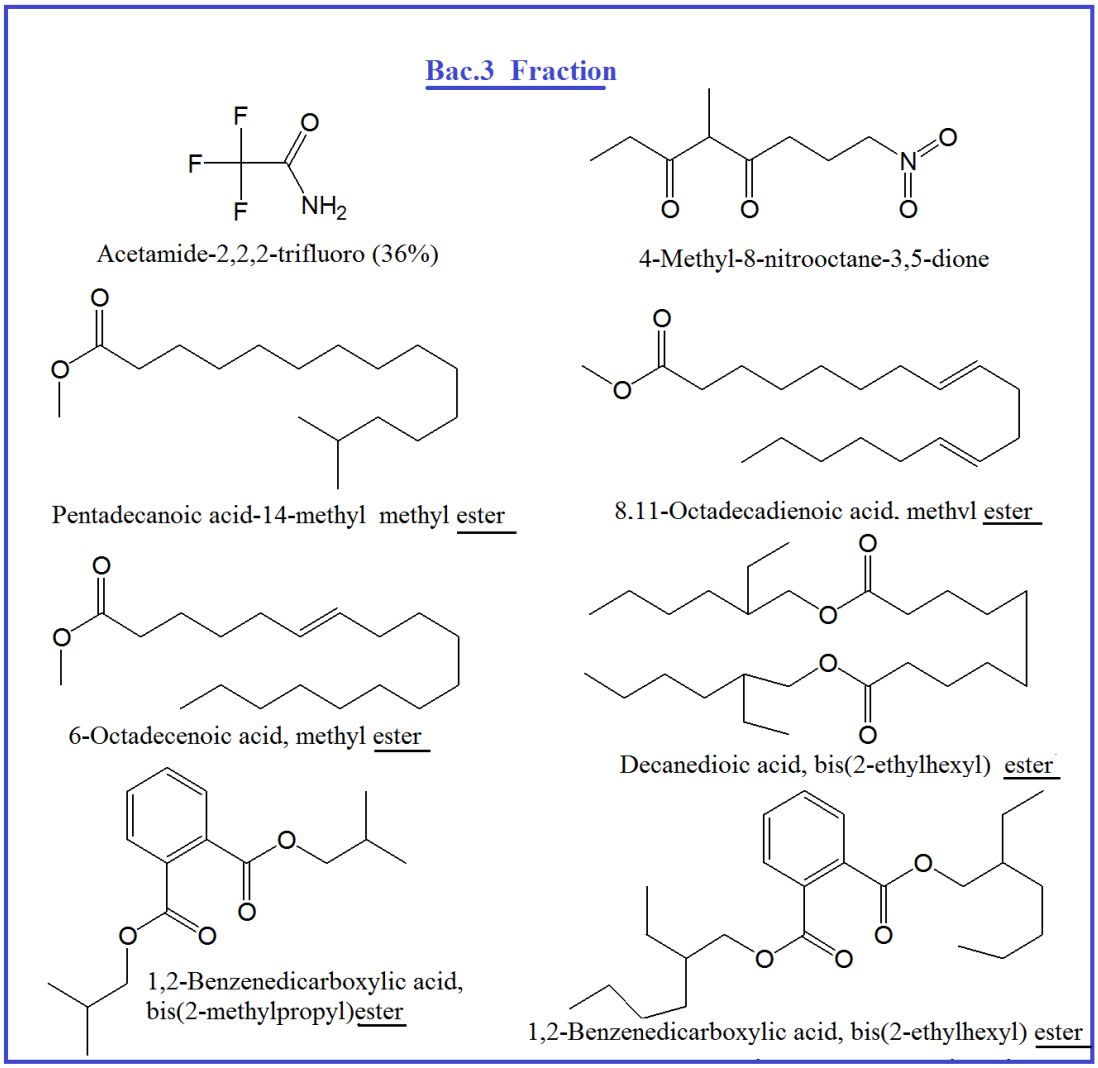


Figure 4. Major Compounds identified by GC/MS analysis in Bac3 fraction

Table 1.

| Nº | $R_t$ (min) | *Fatty acids* | MW | MF | Vib3 (%) | Bac3 (%) |
|---|---|---|---|---|---|---|
| 1 | 21.00 | Propanedioic acid | 248 | $C_9H_{20}O_4Si_2$ | 1.71 | ------ |
| 2 | 42.62 | Tetradecanoic acid | 300 | $C_{17}H_{36}O_2Si$ | 2.20 | ------ |
| 3 | 45.81 | n-Pentadecanoic acid | 314 | $C_{18}H_{38}O_2Si$ | 0.33 | 0.15 |
| 4 | 48.44 | Palmitoleic acid (C16:1) | 326 | $C_{19}H_{38}O_2Si$ | 1.32 | ------ |
| 5 | 49.29 | Palmetic acid (C16:0) | 328 | $C_{19}H_{40}O_2Si$ | **28.95** | ------ |
| 6 | 54.79 | Oleic acid (C18:1) | 354 | $C_{21}H_{42}O_2Si$ | **11.43** | ------ |
| 7 | 54.92 | *cis*-6-Octadecenoicacid (C18:1) | 354 | $C_{21}H_{42}O_2Si$ | **8.43** | ------ |
| | | ***Fatty acids esters*** | | | | |
| 8 | 44.61 | 7-Hexadecenoic acid methyl ester | 268 | $C_{17}H_{32}O_2$ | **0.79** | --- |
| 9 | 45.37 | Pentadecanoic acid-14-methyl methyl ester | 270 | $C_{17}H_{34}O_2$ | 0.26 | 1.63 |
| 10 | 50.67 | 8,11-Octadecadienoic acid, methyl ester | 294 | $C_{19}H_{34}O_2$ | ------ | **3.11** |
| 11 | 50.90 | 6-Octadecenoic acid, methyl ester | 296 | $C_{19}H_{36}O_2$ | ------ | **4.76** |
| 12 | 51.65 | Stearic acid methyl ester | 298 | $C_{19}H_{38}O_2$ | ------ | 0.57 |
| 13 | 69.29 | Decanedioic acid, bis(2-ethylhexyl) ester | 426 | $C_{26}H_{50}O_4$ | ------ | 0.53 |
| | | ***Phenyl compounds*** | | | | |
| 14 | 38.27 | 1(4-Hydroxyphenyl)3-methy-l2-buten-1-one | 176 | $C_{11}H_{12}O_2$ | ------ | 0.32 |
| 15 | 43.32 | 1,2-Benzenedicarboxylic acid,bis(2-methylpropyl)ester | 278 | $C_{16}H_{22}O_4$ | ------ | 0.41 |
| 16 | 46.58 | 1,2-Benzenedicarboxylic acid, dibutyl ester | 278 | $C_{16}H_{22}O_4$ | ------ | 0.13 |
| 17 | 62.11 | Phenol-2,2'-methylenebis[6(1,1--dimethylethyl)4ethyl | 368 | $C_{25}H_{36}O_2$ | ------ | 0.35 |
| 18 | 62.90 | 1,2-Benzenedicarboxylic acid, bis(2--ethylhexyl) ester | 390 | $C_{24}H_{38}O_4$ | ------ | 0.42 |
| | | ***Nitrogenous compounds*** | | | | |
| 19 | 8.63 | Acetamide-2,2,2-trifluoro | 113 | $C_2H_2F_3NO$ | ------ | **36.17** |
| 20 | 22.47 | 4-Methyl-8-nitrooctane-3,5-dione | 201 | $C_9H_{15}NO_4$ | ------ | 0.86 |
| 21 | 49.77 | 2-Isobutoxyquinoline | 201 | $C_{13}H_{15}NO$ | **36.44** | ----- |
| | | ***Sulphur compounds*** | | | | |
| 22 | 6.80 | 5(t-Butyl)-4-methylt-thiophen-2-(5H)one | 170 | $C_9H_{14}OS$ | ---- | 0.32 |
| 23 | 7.41 | 1,4-dithiocyanatobut-2-ene | 170 | $C_6H_6N_2S_2$ | | 0.18 |
| 24 | 34.71 | Aryl sulphonyl-cycloheptatriene | 232 | $C_{13}H_{12}O_2S$ | 0.16 | --- |
| | | ***Terpenes*** | | | | |
| 25 | 6.88 | (1-Acetoxyethyl) bicycle [1.1.1]pentane | 154 | $C_9H_{14}O_2$ | ----- | 0.12 |
| 26 | 30.53 | α-Patchoulene | 204 | $C_{15}H_{24}$ | ---- | 0.09 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 27 | 31.48 | *cis*-α-Bisabolene | 204 | $C_{15}H_{24}$ | | 0.28 |
| 28 | 37.82 | Geranyl-α-terpinene | 272 | $C_{20}H_{32}$ | --- | 0.30 |