Indian J Microbiol (Oct–Dec 2016) 56(4):426–432
DOI 10.1007/s12088-016-0609-1

ORIGINAL ARTICLE

GC–MS Analysis of Bio-active Molecules Derived from *Paracoccus pantotrophus* FMR19 and the Antimicrobial Activity Against Bacterial Pathogens and MDROs

I. Faridha Begum[1] · R. Mohankumar[2] · M. Jeevan[3] · K. Ramani[1]

Received: 1 April 2016 / Accepted: 26 June 2016 / Published online: 20 July 2016

Abstract The present investigation is focused on the study of chemical composition of a bioactive compound derived from a rumen isolate *Paracoccus pantotrophus* FMR19 using GC–MS and to find out the antibacterial activity of the extracted crude bioactive compounds against multidrug resistant organisms (MDROs) and other clinical pathogens. GC–MS analysis revealed that *P. pantotrophus* FMR19 produced eight major compounds that have been reported to exhibit antimicrobial property. The main components identified from hexane fraction are long chain alkanes, fatty alcohols, fatty acid methyl ester and aromatic hydrocarbons. These molecules are not only active against clinical pathogens such as *Salmonella* sp. and *Proteus* sp. and also effective against MDROs such as Metallo β lactamase and Pan drug resistant bacterial strains and Methicillin resistant *Staphylococcus aureus*.

Keywords GC–MS · Bioactive compound · Antibacterial activity · Rumen bacteria · *Paracoccus pantotrophus* FMR19 · Multi-drug resistant organisms

✉ K. Ramani
ramani.k@ktr.srmuniv.ac.in; microramana@yahoo.co.in

[1] Department of Biotechnology, School of Bioengineering, SRM University, Kattankulathur, Tamilnadu 603 203, India

[2] Interdisciplinary Institute of Indian System of Medicine, SRM University, Kattankulathur, Tamilnadu 603 203, India

[3] Department of Microbiology, Sri Muthukumaran Medical College and Research Institute, Chennai, Tamilnadu 600 069, India

Introduction

Bioactive compounds produced by plants and microbes exhibit pharmacological or toxicological effects in man and animals. Bacteria have been considered as one of the significant groups of microorganisms due to their ability to produce a wide array of secondary metabolites, such as antibiotics, antitumor agents, immunosuppressive agents, cosmetics, vitamins, nutritional materials, herbicides, pesticides, anti-parasitic agents and enzymes [1–3]. Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported.

Several novel bioactive compounds have also been discovered from aquatic Actinomycetes [4, 5]: rifamycin, (*Micromonospora* sp.) [6]; salinosporamide-A, an anticancer metabolite (*Salinispora* sp.) [7]; marinomycins (*Marinophilus* sp.) [8]; abyssomicin-C (*Verrucosispora* sp.) and marino pyrroles (*Streptomyces* sp.) [6, 9]. The appearances of multidrug-resistant pathogenic strains caused substantial morbidity and mortality especially among the elderly and immune-compromised patients [10]. To overcome this situation, there is a need to improve or discover a novel class of antibiotics and antimicrobial compounds that have different mechanisms of action worldwide [11].

There have been a number of reports on the antibacterial activities of long-chain fatty alcohols [6]. It has been reported, the activity increases with the length of the carbon chain [12] and the water/octanol partition coefficient has been identified as an important determinant of activity [8, 13]. Other studies indicated that appropriate length of the carbon chain determines the effective activity [14, 15]. However, there is no consensus about the precise length of the carbon chain for the potential activity.

In India, approximately 1,50,000 tonnes of offals in the form of rawhide trimmings, limed animal fleshing, green animal fleshing, hide splits and chrome shavings are disposed, that are not utilized or underutilized thus creating a solid waste disposal problem in tanneries [16]. In anaerobic bacterial fermentation, the proteins and amino acids derived from degradation of animal fleshing results in branched chain fatty acid, accompanied by potentially important metabolites such as amines, phenolic compounds, and volatile sulfur compounds.

The present work focuses on the utilization of animal fleshing (ANFL), as a substrate for the microbial production of the bioactive compounds. The ability of microorganisms to grow and produce an appreciable level of bioactive compounds, using ANFL as substrate could offer tremendous potential for the development of biotechnological methods for the rapid hydrolysis of ANFL. The produced crude bioactive preparation was characterized using GC–MS analysis and used as an antibacterial agent against multidrug resistant organisms (MDROs) and clinical pathogens.

Materials and Methods

Isolation of Rumen Microbes

The rumen fluid collected from the slaughter house was filtered through double layer muslin cloth. One ml of filtrate was serially diluted and cultured by spread plate using Hungate's medium (HiMedia) under anaerobic conditions. The isolated microbe was identified as *Paracoccus pantotrophus* and the 16S rRNA sequence obtained was submitted to NCBI GenBank with the assigned number as JX012237.

Production of Bioactive Compound

The rumen bacterial strain was inoculated in anaerobic basal medium namely Hungate's broth containing the composition (g/L): (KH_2PO_4—0.02 g; K_2HPO_4—0.03 g; $MgSO_4$—0.01 g; $CaCl_2$—0.01 g; $(NH_4)_2SO_4$—0.1 g; NaCl—0.1 g; Cysteine HCl—0.02 g; $NaHCO_3$—0.5 g; resazurin—0.0001 g; cellulose—1.0 g; Trace element-1 ml/l). In addition to that, the medium was supported with 1:4 ratio of cellulose and leather industry solid waste animal flesh and incubated for 9 days at 37 °C to achieve the better degradation of animal flesh and thereby the high yield of bioactive compounds. The samples were centrifuged at $10{,}000 \times g$ in 10 min and the cell free supernatant was collected and extracted with chloroform. The hexane fraction that showed good antibacterial effect was subjected to GC–MS analysis to identify the nature of compounds. The chloroform was removed using rotary evaporator under reduced pressure to get crude extract and the crude extract was further purified by column chromatography. A glass column of 50 cm height and 3 cm diameter with silica gel (100–200 Mesh size) was used for the purification of the crude extract. The admixture is loaded at top of the column and the fractions were eluted using non polar solvents (hexane or ethyl acetate).

Antibacterial Bioassay

The antibacterial activity of isolated fractions was checked against clinical pathogens such as *Proteus* sp.,

Table 1 GC–MS conditions

GC programme	
Column	HP-5MS (5 % phenyl methyl siloxane), 30 m × 250 µm × 0.25 µm
Equipment	Agilent Technologies (GC-7890B: MS-5977AMSB)
Carrier gas	Helium gas 1 ml/min, splitless mode
Detector	Mass detector
Software	MassHunter
Sample injection	1 µl
Oven temperature programme	35 °C (2 min hold) up to 275 °C at the rate of 2 °C/min on hold
Injection temperature	200 °C
Total GC run time	122 min
MS programme	
Library used	NIST version—2011
Inlet line temperature	200 °C
Source temperature	300 °C
Electron energy	70 eV
Mass scan (m/z)	40–700 amu
Solvent delay	2 min
Total MS run time	120 min

Table 2 GC–MS analysis of the crude hexane fraction

S. no.	Compound name	Retention time (min)	Formula	Molecular weight	Area %	Activity	Compound nature
1	1-Dodecene	29.471	$C_{12}H_{24}$	168.31	1.624	Antibacterial [22]	Long chain alkene
2	Dodecane	30.032	$C_{12}H_{26}$	170.34	0.339	Anti-oxidant [26]	Long chain alkane
3	Benzene, 1,3-bis(1,1-dimethylethyl)-	33.505	$C_{14}H_{22}$	190.32	0.396	Antibacterial activity [28]	Aromatic hydro carbon
4	2,4-Dimethyldodecane	34.369	$C_{14}H_{30}$	198.38	0.146	Antimicrobial and antioxidant activity [24]	Long chain alkane
5	Dodecane, 2,6,11-trimethyl-	35.461	$C_{15}H_{32}$	212.41	0.417	Antibacterial activity [24]	Alkane
6	n-Tridecan-1-ol	42.861	$C_{13}H_{28}O$	196.37	6.861	Antibacterial activity [30]	long-chain fatty alcohol
7	Tetradecane	43.285	$C_{14}H_{30}$	198.39	0.954	Antimicrobial diuretic, anti tuberculosis [27]	Long chain alkane
8	Tetradecane, 4-methyl-	44.604	$C_{15}H_{32}$	212.41	0.135	Antimicrobial activity [24]	Hydro carbon
9	Pentadecane, 2,6,10-trimethyl-	46.500	$C_{18}H_{38}$	254.49	0.171	Antimicrobial activity [24]	Hydro carbon
10	Heptadecane	48.638	$C_{17}H_{36}$	240.46	0.151	Antimicrobial activity [24]	Hydro carbon
11	Pentadecane	49.396	$C_{15}H_{32}$	212.41	0.241	Antimicrobial and antioxidant activity [27]	Hydro carbon
12	Phenol, 2,4-bis(1,1-dimethylethyl)-	50.169	$C_{14}H_{22}O$	206.32	1.115	Antibacterial activity [26, 16]	Aromatic hydro carbon
13	Benzoic acid, 4-ethoxy-, ethyl ester	50.761	$C_{11}H_{14}O_3$	194.22	0.139	Antimicrobial preservative [33]	Aromatic acid ester
14	Dodecane, 2,6,11-trimethyl-	51.792	$C_{15}H_{32}$	212.41	0.351	Antibacterial activity [24]	Alkane
15	Hexadecane	55.249	$C_{16}H_{34}$	226.44	0.806	Antimicrobial and antioxidant activity [16]	Long chain hydrocarbon
16	Eicosane	57.993	$C_{20}H_{42}$	282.54	0.250	Antibacterial activity [20]	Long chain fatty acid
17	1,11-Tridecadiene	60.359	$C_{13}H_{24}$	180.329	0.326	Antimicrobial activity [34]	Alkene
18	E-15-Heptadecenal	65.818	$C_{17}H_{32}O$	252.43	10.555	Antioxidant and antibacterial activity [16]	Long chain alkene
19	*tert*-Hexadecanethiol	67.091	$C_{16}H_{34}S$	258.51	0.887	Antioxidant and antibacterial activity [37]	Long chain thiol
20	Isopropyl myristate	67.440	$C_{17}H_{34}O_2$	270.45	0.422	Antioxidant and antibacterial activity [23]	Long chain fatty ester
21	Hexadecanoic acid, methyl ester	72.383	$C_{17}H_{34}O_2$	270.45	0.706	antibacterial, antioxidant, antitumor, immunostimulant, chemopreventive and lipoxygenase inhibitor [24]	Long chain fatty ester
22	3-Pentadecanone	73.551	$C_{15}H_{30}O$	226.39	0.185	Antioxidant and antibacterial activity [36]	Long chain ketone
23	Heneicosane	74.157	$C_{21}H_{44}$	296.57	0.288	Antiasthmatics urine acidifiers Antimicrobial [26]	Hydro carbon
24	*n*-Nonadecanol-1	75.674	$C_{19}H_{40}O$	284.52	7.956	Antimicrobial and cytotoxic properties [35]	Long chain alcohol
25	8-Octadecenoic acid, methyl ester	80.389	$C_{19}H_{36}O_2$	296.487	0.736	Antioxidant, antimicrobial [24]	Fatty acid ester
26	Methyl stearate	81.648	$C_{19}H_{38}O_2$	298.28	0.288	Antidiarrheal 19 and cytotoxic and antiproliferative Activities [35]	Fatty acid methyl Esters
27	Tetracosane	81.921	$C_{24}H_{50}$	338.65	0.553	Antioxidant and antimicrobial activity [20]	Alkane
28	Oxiraneoctanoic acid	88.668	$C_{19}H_{36}O_3$	186.24	0.945	Antioxidant activity [32]	Fatty acid
29	*n*-Tetracosanol-1	92.929	$C_{24}H_{50}O$	354.65	4.641	Antibacterial activity [30]	Alcoholic compound
30	6,9,12-Octadecatrienoic acid, methyl ester	95.386	$C_{19}H_{32}O_2$	292.45	0.315	Antibacterial activity [24]	Polyenoic fatty acid methyl ester
31	Octadecanoicacid, 9,10-dichloro-, methyl ester	97.084	$C_{19}H_{36}Cl_2O_2$	367.40	4.166	Antibacterial activity [24]	Unsaturated fatty acid
32	1-Heptacosanol	100.572	$C_{27}H_{56}O$	396.73	2.738	Antimicrobial and anti-oxidant activity [31]	Long chain alcohol

Table 2 continued

S. no.	Compound name	Retention time (min)	Formula	Molecular weight	Area %	Activity	Compound nature
33	17-Pentatriacontene	102.603	$C_{35}H_{70}$	490.93	0.422	Antioxidant activity [16]	Alkene
34	Tetratriacontane	104.317	$C_{34}H_{70}$	478.92	0.401	Antibacterial activity [30]	Long chain Hydrocarbon
35	1-Dodecanol	114.325	$C_{20}H_{42}O$	186.33	0.676	Anti-oxidant, antibacterial activity [25]	long-chain fatty alcohol
36	Hentriacontane	117.585	$C_{31}H_{64}$	436.85		Antibacterial activity [29]	Long chain hydrocarbon

Salmonella sp., *Salmonella paratyphi* A, *Salmonella paratyphi* B and *Staphylococcus aureus*. Activity was also tested against MDROs such as Metallo β-lactamase resistant organism (MBL)-*Pseudomonas aeruginosa*, Pan drug resistant organism (PDR)—*Klebsiella pneumoniae* and Methicillin Resistant *S. aureus* (MRSA). These cultures were obtained from Sri Muthukumaran Medical College, Chennai. The multidrug resistance was confirmed by agar well diffusion method. Briefly, the bacterial lawn (10^{-8} CFU/ml) of the each test organism was prepared in Mueller–Hinton agar plate. The agar plates were allowed to dry and wells of 10 mm were made with a sterile cork borer on the inoculated agar plates. 10 µg of the active fraction in Dimethyl sulfoxide (DMSO) was added into the well and the DMSO was used as control. The plates were incubated at 37 °C for 24 h and observed for the zone of inhibition around the wells and the zone of inhibition was measured using an antibiotic zone scale (Himedia, Mumbai).

GC–MS Analysis of the Crude Bioactive Compound in Hexane Fraction

The crude bioactive hexane fraction was subjected to GC–MS analysis (Fig. 1) and the conditions used for the GC–MS analysis are presented in Table 1. The spectrum of the crude component was compared with the spectrum of the known components in the National Institute Standard and Technology (NIST) library [17]. The name, molecular formula, weight and chemical structure of the components of the test materials were identified.

Results and Discussion

Production of Antibacterial Compound and GC–MS Analysis

Paracoccus pantotrophus FMR19 produced eight major compounds, identified from hexane fraction by GC–MS analysis as long chain alkanes [E-15 Heptadecanol], fatty alcohols [n-Nonadecanol-1, 7.956 %; n-Tridecan-1-ol, 6.861 %; n-tetracosanol-1, 4.641 %; 1-Heptacosanol, 2.738 %], fatty acid methyl ester [Octadecanoic acid, 9,10-dichloro-, methyl ester, 4.166 %] and aromatic hydrocarbon [2,6-bis(1,1-dimethylethyl)phenol, 1.115 %] and other minor compounds are also represented in Table 2. These major compounds may be responsible for antibacterial activity [6, 7, 12, 15].

Retention time at 65.818 min corresponds to the compound E-15-Heptadecenol with peak area 10.555 %. Long chain alkanes such as Hexadecane have also been reported to have antibacterial and antioxidant activities [15].

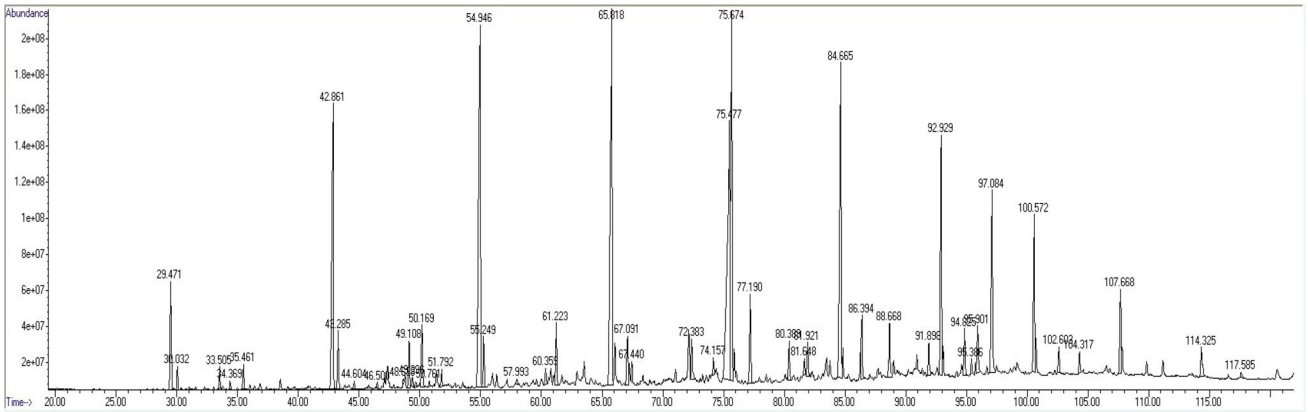

Fig. 1 GC–MS chromatogram of the hexane fraction of the crude compounds from *Paracoccus pantotrophs*

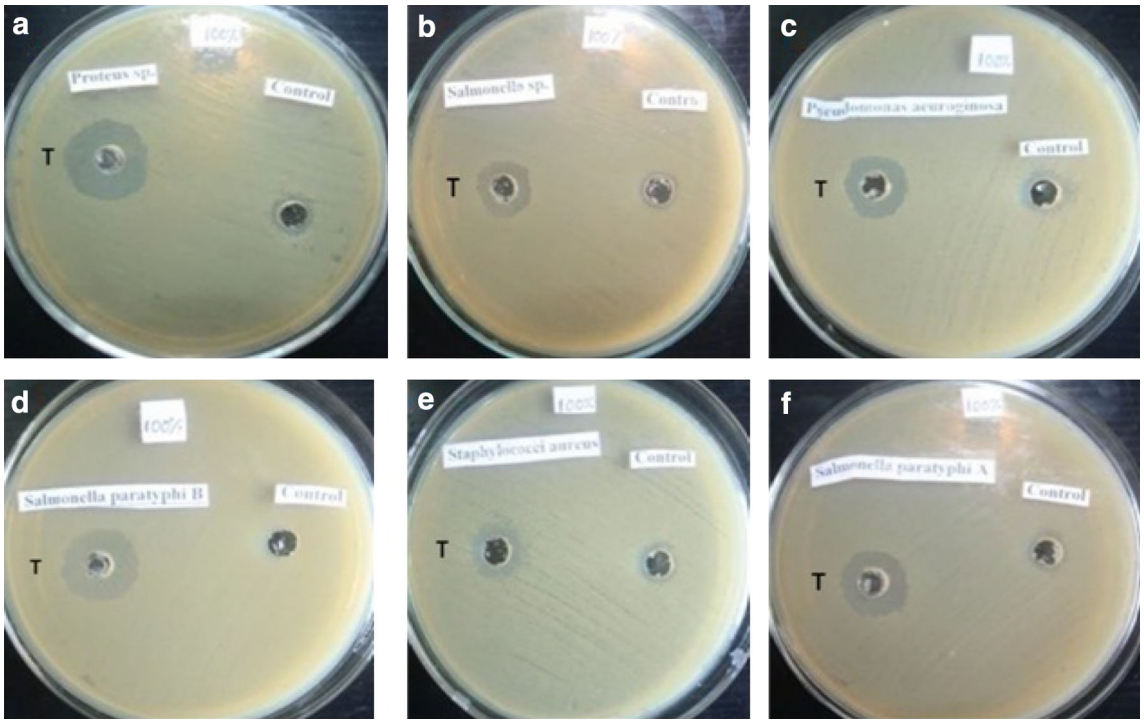

Fig. 2 Zone of inhibition against microbial pathogens such as **a** *Proteus* sp., **b** *Salmonella* sp., **c** *Pseudomonas aeruginosa*, **d** *Salmonella paratyphi* B, **e** *Staphylococcus aureus* and **f** *Salmonella paratyphi* A. Control: DMSO; *T* test: 10 µg of crude compounds in DMSO

The n-Nonadecanol-1 compound appeared at 75.674 min with a peak area 7.956 % and has been proven to have cytotoxic property and antibacterial activity [1]. The compound 1-Heptacosanol appeared at 100.572 min with a peak area of 2.738 % are reported to have antimicrobial and antioxidant activity [2].

The crude bioactive compound produced in the study contains fatty acid methyl esters. Only few studies have been reported that the crude bioactive compound showed the antimicrobial activity against *Bacillus subtilis* and *Sarcina lutea*, but there is no antimicrobial activity against the growth of *S. aureus* and *Pectobacterium carotovorum* at a concentration of 2000 µg/ml. Whereas the compound extracted in our study showed antibacterial activity against *S. aureus* (4 mm) proving to be even more effective at a concentration of 10 µg/ml. Fatty acid methyl esters from microalgae were also reported to possess antimicrobial properties [18, 19]. Also, few aromatic hydrocarbons are shown to have antibacterial activity against the microbial pathogens [20, 21]. Hence, all those components eluted in hexane might be attributed to the antimicrobial activity of the MDROs and clinical pathogens.

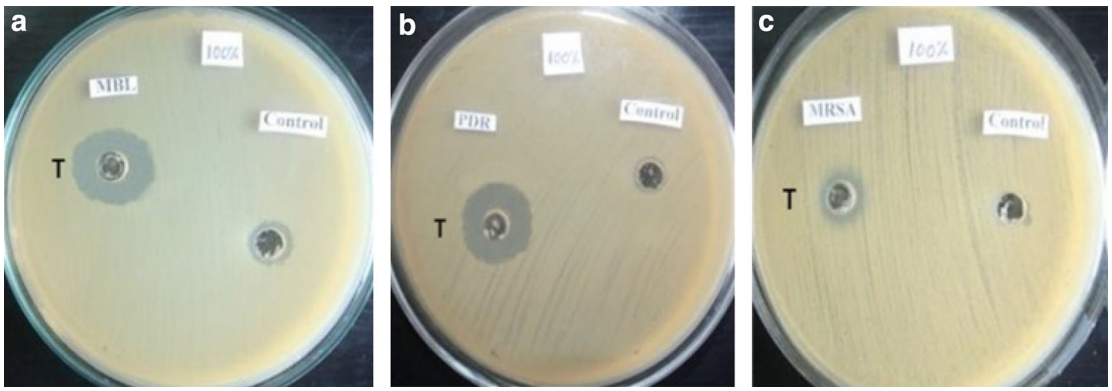

Fig. 3 Zone of inhibition against multi drug resistant organisms such as **a** MBL, **b** PDR, and **c** MRSA. Control: DMSO; *T* test: 10 μg of crude compounds in DMSO

Antibacterial Activity of the Bioactive Molecule Extracted in Hexane

The clinical pathogens *Proteus* sp. (7 mm), *Salmonella* sp. (5 mm), *Pseudomonas aeruginosa* (5 mm), *Salmonella paratyphi*-B (6 mm), *S. aureus* (4 mm), *Salmonella paratyphi*-A (5 mm) showed zone of inhibition. The bioactive hexane fraction showed 8, 7 and 4 mm zones of inhibition against metallo β lactamase resistant (MBL) bacterial strain, pan-drug resistant (PDR) bacterial strain and methicillin-resistant *S. aureus* (MRSA) respectively. The results showed that the hexane fraction is more active against MDROs than the clinical pathogens (Figs. 2, 3).

Naoko et al. [22] reported that the long chain fatty acids 1-Dodecanol, n-Nonadecanol-1, and 1-tridecanol had the highest antibacterial activity against *S. aureus*. Chandrasekar et al. [23] showed that NimbapatradiChoornam had strong antimicrobial activity against *Klebsiella pneumoniae*, *S. aureus*, *E. coli* and *Candida albican.* The presence of phenol, 2,4-bis[1,1-dimethylethyl]-derivative, isopropyl myristate, eicosane, octadecanoic acid and hexadecanoic acid compounds were responsible for the antimicrobial activity of Nimbadipatrachoornam.

Rahbar et al. [24] had done investigation on leaf and stem extracts of *Origanumvulgare L.* sp. and found good antimicrobial activity of tridecane, 9, 12, 15-octadecatrienoic acid [ω-3], tetradecane, hexadecanoic acid and pentadecane against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *S. aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), as well as three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*).

Acknowledgments The author I. Faridha Begum, Senior research Fellow is grateful for the financial assistance by University Grant Commision.

References

1. Atta HM, Dabour SM, Desoukey SG (2009) Sparsomycin antibiotic production by *Streptomyces* sp. AZ-NIOFD1: taxonomy, fermentation, purification and biological activities. Am Eurasian J Agric Environ Sci 5:368–377
2. Imada C (2005) Enzyme inhibitors and other bioactive compounds from marine *Actinomycetes*. Antonie Van Leeuwenhoek 87:59–63. doi:10.1007/s10482-004-6544-x
3. Valli S, Suvathi SS, Aysha OS, Nirmala P, Vinoth KP, Reena A (2012) Antimicrobial potential of *Actinomycetes* species isolated from marine environment. Asian Pac J Trop Biomed 2:469–473. doi:10.1016/S2221-1691(12)60078-1
4. Solanki R, Khanna M, Lal R (2008) Bioactive compounds from marine *Actinomyces*. Indian J Microbiol 48:410–431. doi:10.1007/s12088-008-0052-z
5. Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31:224–245. doi:10.1016/j.biotechadv.2012.10.004
6. Hughes CC, Prieto-Davo A, Jensen PR, Fenical W (2008) The marinopyrroles, antibiotics of an unprecedented structure class from a marine *Streptomyces* sp. Org Lett 10:629–631. doi:10.1021/ol702952n
7. Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W (2003) Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora*. Angew Chem Int Ed Engl 42:355–357. doi:10.1002/anie.200390115
8. Jensen PR, Gontang E, Mafnas C, Mincer TJ, Fenical W (2005) Culturable marine *Actinomycete* diversity from tropical Pacific Ocean sediments. Environ Microbiol 7:1039–1048. doi:10.1111/j.1462-2920.2005.00785.x
9. Riedlinger J, Reicke A, Zahner H, Krismer B, Bull AT, Maldonado LA, Ward AC, Goodfellow M, Bister B, Bischoff D, Sussmuth RD, Fiedler HP (2004) Abyssomicins, inhibitors of the para-aminobenzoic acid pathway produced by the marine *Verrucosispora* strain AB-18-032. J Antibiot 57:271–279. doi:10.1038/ja.2007.54
10. Koul S, Jyotsana P, Anjali M, Kalia VC (2016) Potential emergence of multi-quorum sensing inhibitor resistant (MQSIR) bacteria. Indian J Microbiol 56:1–18. doi:10.1007/s12088-015-0558-0
11. Mukherjee K, Tribedi P, Mukhopadhyay B, Sil AK (2013) Antibacterial activity of long-chain fatty alcohols against *Mycobacteria*. FEMS Microbiol Lett 338:177–183. doi:10.1111/1574-6968.12043

12. Kabelitz N, Santos PM, Heipieper HJ (2003) Effect of aliphatic alcohols on growth and degree of saturation of membrane lipids in *Acinetobacter calcoaceticus*. FEMS Microbiol Lett 220:223–227. doi:10.1016/S0378-1097(03)00103-4
13. Willis AT (2014) Anaerobic bacteriology: clinical and laboratory practice. Butterworth-Heinemann, London
14. Rajalakshmi S, Mahesh N (2014) Production and characterization of bioactive metabolites isolated from *Aspergillus terreus* in rhizosphere soil of medicinal plants. Int J Curr Microbiol Appl Sci 3:784–798
15. Kalpana Devi V, Shanmugasundaram R, Mohan VR (2012) GC–MS analysis of ethanol extracts of *Entada pursaetha* dc seed. Biosci Discover 3:30–33
16. Yogeswari S, Ramalakshmi S, Neelavathy R, Muthumary J (2012) Identification and comparative studies of different volatile fractions from *Monochaetia kansensis* by GCMS. Glob J Pharmacol 6:65–71
17. Chaudhary R, Tripathy A (2015) Isolation and identification of bioactive compounds from Irpex Lacteus Wild Fleshy Fungi. J Pharm Sci Res 7:424–434
18. Salem MZ, Ali HM, Mansour MM (2014) Fatty acid methyl esters from air-dried wood, bark, and leaves of *Brachychiton diversifolius* R. Br: antibacterial, antifungal, and antioxidant activities. Bioresources 9:3835–3845
19. Suresh A, Praveenkumar R, Thangaraj R, Oscar FL, Baldev E, Dhanasekaran D, Thajuddin N (2014) Microalgal fatty acid methyl ester a new source of bioactive compounds with antimicrobial activity. Asian Pac J Trop Dis 4:979–984. doi:10.1016/S2222-1808(14)60769-6
20. Boussaada O, Ammar S, Saidana D, Chriaa J, Chraif I, Daami M, Helal AN, Mighri Z (2008) Chemical composition and antimicrobial activity of volatile components from capitula and aerial parts of *Rhaponticum acaule* DC growing wild in Tunisia. Microbiol Res 163:87–95. doi:10.1080/10412905.2009.9700142
21. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253. doi:10.1016/j.ijfoodmicro.2004.03.022
22. Naoko T, Akiko S, Miki N, Keisuke M, Kazutoyo E, Hajime H, Yoshihiro I (2007) Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. Molecule 12:139–148. doi:10.3390/12020139
23. Chandrasekar T, Rao MRK, Kumar RV, Prabhu K, NandhaKumar V, Divya D (2015) GC–MS analysis, antimicrobial, antioxidant activity of an Ayurvedic medicine. Nimbapatradi Choornam. J Chem Pharm Res 7:124–136. doi:10.1155/2014/694934
24. Rahbar N, Shafagha A, Salimi F (2012) Antimicrobial activity and constituents of the hexane extracts from leaf and stem of *Origanum vulgare* L. sp. Viride (Boiss.) Hayek. Growing wild in Northwest Iran. J Med Plants Res 6:2681–2685. doi:10.5897/JMPR11.1768
25. Raj Kumar K, Brindha Priyadarisini V, Ranjith Kumar M (2012) Isolation and identification of bioactive compounds from Bacillus Megateriume5 from the south east coastal region of India against urinary tract infectious pathogens. Int J Phar Biol Archives 3:842–847
26. Usha Nandhini S, Sangareshwari S, Lata K (2015) Gas chromatography-mass spectrometry analysis of bioactive constituents from the marine *Streptomyces*. Asian J Pharm Clin Res 8:244–246
27. Girija S, Veeramuthu D, Pandi Suba K, Hariprasad G, Raghuraman R (2014) Chromatographic characterization and GC-MS evaluation of the bioactive constituents with antimicrobial potential from the pigmented ink of *Loligo duvauceli*. Int Sch Res Notices. doi:10.1155/2014/820745
28. Al-Youssef HM, Hassan WHB (2015) Antimicrobial and antioxidant activities of *Parkinsonia* aculeata and chemical composition of their essential oils. Merit Res J Med Med Sci 3:147–157
29. Olubunmi A, Gabriel OA, Stephen AO, Scott FO (2011) Antioxidant and antimicrobial activity of cuticular wax from *Kigelia Africana*. FABAD J Pharm Sci 34:187–194
30. Garaniya N, Bapodra A (2014) Ethno botanical and Phytophrmacological potential of *Abrus precatorius L.*: a review. Asian Pac J Trop Biomed 4:27–34. doi:10.12980/APJTB.4.2014C1069
31. Al-Abd NM, Mohamed ZN, Mansor M, Azhar F, Hasan MS, Kassim M (2015) Antioxidant, antibacterial activity, and phytochemical characterization of *Melaleuca cajuputi* extract. BMC Complement Altern Med 15:1–13. doi:10.1186/s12906-015-0914-y
32. Nahar N, Rahman S, Rahman SM, Moniruzzaman M (2016) GC-MS analysis and antibacterial activity of *Trigonella foenum-graecum* against bacterial pathogens. Free Radical Antioxid 6:109–114. doi:10.5530/fra.2016.1.13
33. Hussain AZ, Kumaresan S (2014) GC-MS studies and phytochemical screening of *Sesbania grandiflora L.* J Chem Pharm Res 6:43–47
34. Afolayan AJ, Ashafa AOT (2009) Chemical composition and antimicrobial activity of the essential oil from *Chrysocoma ciliata L.* leaves. J Med Plant Res 3:390–394
35. Kuppuswamy MK, Jonnalagadda B, Arockiasamy S (2013) GC-MS analysis of chloroform extract of *Croton bonplandianum*. Int J Pharm Bio Sci 4:613–617
36. Bhardwaj A, Shakil NA, Jha V, Gupta RK (2014) Screening of nutritional, phytochemical, antioxidant and antibacterial activity of underutilized seeds of *Scirpus articulatus*: the basis of Khubahi Ramdana industry. J Pharma Phytochem 3:311–320
37. Sivakumar SR (2014) GC-MS analysis and antibacterial potential of white crystalline solid from red algae *Portieria hornemannii* against the plant pathogenic bacteria *Xanthomnas axonopodis pv. citri* (Hasse) Vauterin et al. and *Xanthomonas campestris pv. malvacearum* (smith 1901) dye 1978b. Int J of Adv Res 2:174–183